INFECTION BY DRY, AIRBORNE *BOTRYTIS CINEREA* CONIDIA AND FUNGICIDE EFFICACY ON DIFFERENT PARTS OF GRAPE BUNCHES AND VINELETS

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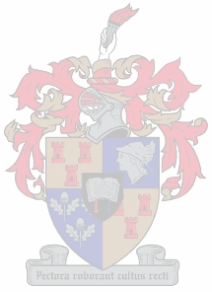

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Supervisor: Prof. G. Holz
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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

INFECTION BY DRY, AIRBORNE *BOTRYTIS CINEREA* CONIDIA AND FUNGICIDE EFFICACY ON DIFFERENT PARTS OF GRAPE BUNCHES AND VINELETS

SUMMARY

The evaluation of fungicide efficacy in commercial vineyards can be influenced by the sporadic occurrence of *Botrytis cinerea* at various positions on vines, differences in bunch structure during bunch development and the phenomenon that symptom expression in shoots and bunches is governed by the resistance reaction of the various shoot and bunch parts. It has been postulated that, following air and water dispersal, infection by solitary conidia should play a prominent role in the epidemiology of *B. cinerea* on grapevine. The aim of this study was to determine (i) infection and (ii) fungicide efficacy at specific sites on shoots of vinelets and bunches (table grape cultivar Dauphine and the wine grape cultivar Merlot) inoculated with dry, airborne conidia of *B. cinerea*.

Vinelets, prepared from cuttings, and bunches obtained from the vineyards at full bloom, pea size, bunch closure, véraison and harvest stages, were sprayed in a spray chamber at the recommended dosages with iprodione, pyrimethanil, cyprodinil/fludioxonil and fenhexamid or were left unsprayed. After 24 h the vinelets or bunches were dusted with dry conidia of *Botrytis cinerea* in a settling tower and incubated for 24 h at a high relative humidity (±93%). Following incubation, both the vinelets or bunches were divided into three groups. Vinelets and bunches of the one group were surface-sterilised, the others were left unsterile. Vinelets and bunches of one unsterile group were placed in dry chambers, kept for 14 days at 22°C with a 12 h photoperiod daily and monitored for symptom expression and the development of *B. cinerea*. Vinelets and bunches of the sterile group, and from one unsterile group were used for isolation. From each of these vinelets leaf blades, leaf petioles, shoots and inflorescences were removed. Sites used for isolation in bunch parts were rachises, laterals and pedicels, and sites on berries were the pedicel-end, cheek and style-end. The different parts and segments were placed in Petri dishes on Kerssies' *B. cinerea* selective medium, or on water agar medium supplemented with paraquat and incubated for 14 days at 22°C with a 12 h photoperiod daily. Infection and fungicide efficacy was determined by observing intact

vinelets and bunches for symptom expression, and by estimating the amount of *B. cinerea* at the various sites on the vinelets and bunches with isolation studies. No symptoms of *B. cinerea* decay developed on sprayed and unsprayed vinelets that were kept in dry chambers during the 2 wk observation period. The isolation and incubation studies showed that the different fungicides were highly and nearly equally efficient in reducing superficial *B. cinerea* inoculum and latent infection. In the case of leaf blades, which showed a high amount of *B. cinerea* on unsprayed vinelets under the two sterility regimes, decay was significantly reduced by each fungicide on both cultivars. This was not the case for the other parts, which yielded *B. cinerea* at low incidences under the two sterility regimes.

The study with bunches showed that dry, airborne conidia, and the fungicide sprays, penetrated loose and tight clustered bunches from bloom to harvest and evenly landed on the various bunch parts. At full bloom, the amount of *B. cinerea* in unsprayed bunches was high on the laterals and pedicels, but low on the embryos. Unsprayed intact bunches at full bloom were highly susceptible to *B. cinerea* and developed symptoms of grey mould. The fungicides inhibited symptom expression at full bloom, but could not prevent infection. Unsprayed bunches inoculated at the other stages remained asymptomatic. The amount of *B. cinerea* was generally high in the rachises and laterals at pea size and bunch closure stages, and in the pedicel end of berries at harvest. Infection was constantly low in the berry cheek. The fungicides had a differential effect on infection at the various sites. In the case of rachises, the amount of *B. cinerea* was at each growth stage drastically reduced by each fungicide. In laterals, it was effectively reduced at pea size and bunch closure. However, at these two sites, significant differences were found between the fungicides in efficacy at stages when the amount of *B. cinerea* was high. This study showed that if these fungicides are applied properly to vine in commercial vineyards between budding and prebloom, during flowering, and at bunch closure, they should effectively prevent infection and symptom expression and thus the development of *B. cinerea* epiphytotics.

INFEKSIE DEUR DROË, LUGGEDRAAGDE *BOTRYTIS CINEREA* KONIDIA EN DIE EFFEK VAN FUNGISIEDE OP VERSKILLENDE SETELS BINNE WINGERDTROSSE EN OP LOTE

OPSOMMING

Evaluering van fungisieddoeltreffendheid in kommersiële wingerde word beïnvloed deur die sporadiese voorkoms van *Botrytis cinerea* op verskeie posisies van wingerddele, verskille in trosstruktuur tydens trosontwikkeling, en die feit dat simptoomuitdrukking in lote en trosse deur die weerstandsaksie van die verskillende morfologiese dele van lote en trosse beheer word. In die natuur speel infeksie deur enkel konidia 'n prominente rol in die epidemiologie van *B. cinerea* van wingerd. Die doel van hierdie studie was om (i) infeksie en (ii) die effek van fungisiede op verskillende posisies op lote en trosse (tafeldruif kultivar Dauphine, wyndruif kultivar Merlot), wat met droë, luggedraagde konidia van *B. cinerea* geïnokuleer is, te bepaal.

Lote, verkry vanaf steggies, en trosse versamel vanuit die wingerde tydens blom-, ertjiekorrel-, trostoemaak-, deurslaan- en oesstadium, is teen aanbevole dosisse met iprodione, pyrimethanil, cyprodinil/fludioxonil of fenhexamid in 'n spuitkas bespuit, of is onbehandeld gelaat. Na 24 h is die lote en trosse met droë konidia van *B. cinerea* in 'n inokulasietoring geïnokuleer en daarna vir 24 h onder hoë humiditeit [±93% RH] geïnkubeer. Na inkubasie is die lote en trosse in drie groepe verdeel. Die een groep lote en trosse is oppervlakkig gesteriliseer om die patogeen op die oppervlakte te elimineer, en die ander twee groepe is onbehandeld gelaat. Die lote en trosse van een nie-steriele groep is vir 14 dae in droë voghokke by 22°C met 'n 12 uur daaglikse fotoperiode geplaas, en daagliks vir siekte-uitdrukking en die ontwikkeling van *B. cinerea* gemonitor. Lote en trosse van die ander twee groepe is vir isolasiestudies gebruik. Vanaf elke loot is blaarskywe, blaarstele, internodes en ongeopende blomtrossies verwyder. Vanaf trosse is ragisse, laterale en korrelstele verwyder, en vanaf korrels is skilsegmente aangrensend aan die korrelsteel, die stempel-end, en die wang verwyder. Die dele en segmente is op *B. cinerea* selektiewe medium, en op paraquat medium in Petri bakkies geplaas en vir 14 dae by 22°C met 'n 12 uur daaglikse fotoperiode geïnkubeer. Infeksie en die fungisiedeffek is bepaal deur die intakte lote en trosse vir siekte-

uitdrukking te monitor, en deur die hoeveelheid *B. cinerea* op verskeie posisies op lote en trosse te bepaal. Geen simptome het op enige posisie op bespuite en onbespuite lote, wat in droë hokke gehou is, ontwikkel nie. Die isolasie- en inkubasiestudies het getoon dat die verskillende fungisiede hoogs effektief op lote was, en inokulumvlakke van die patogeen doeltreffend verlaag het. In die geval van blaarskywe, wat hoë vlakke van *B. cinerea* op onbespuite steggies onder die twee steriliteitskondisies getoon het, is verrotting op beide kultivars betekenisvol deur die fungisiedes verlaag. Dit het egter nie vir die ander dele, waarop daar 'n lae voorkoms van *B. cinerea* onder die twee steriliteitskondisies was, gegeld nie.

Die studie met trosse het getoon dat droë, luggedraagde konidia en fungisiednewels beide oop en kompakte trosse vanaf blomstadium tot oes penetreer en eweredig op die verskillende dele land. Met blomstadium was die hoeveelheid *B. cinerea* in onbespuite trosse hoog op laterale en korrelstele, maar laag op die embrios. Onbespuite, intakte trosse was hoogs vatbaar vir *B. cinerea* by blomstadium en het simptome van vaalvrot ontwikkel. Die fungisiede het siekte-uitdrukking by blomstadium voorkom, maar kon nie infeksie voorkom nie. Onbespuite trosse wat op ander stadia geïnokuleer is, het geen siekte-uitdrukking getoon nie. Die hoeveelheid *B. cinerea* was hoër in die ragi, asook in laterale by ertjiekorrel- en trostoemaak stadium, en hoër in korrelstele by oesstadium. Infeksie was konstant laag in die korrelskil. Die fungisiede het 'n differensiële effek op infeksie by die verskillende posisies gehad. In die geval van ragi was die hoeveelheid *B. cinerea* drasties deur elke fungisied by alle groeistadia verlaag. In laterale was dit effektief by ertjiekorrel- en trostoemaakstadium verminder. By hierdie twee posisies waar die hoeveelheid *B. cinerea* hoog was, is daar egter betekenisvolle verskille in die doeltreffendheid van fungisiedes gevind. Hierdie studie toon dat as fungisiede behoorlik in kommersiële wingerde tussen botvorming en blomstadium, en tydens blom- en trostoemaakstadium toegedien word, infeksie en siekte-uitdrukking, en dus ook die epifitotiese ontwikkeling van *B. cinerea,* voorkom behoort te word.

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CONTENTS

1. INFECTION AND FUNGICIDE EFFICACY AGAINST *BOTRYTIS CINEREA* ON GRAPEVINE

INTRODUCTION

Botrytis cinerea Pers.: Fr., a pathogen of grapevine (*Vitis vinifera* L.), can infect most of the plant's organs. The pathogen attacks bunches, leaves, buds and canes of grapevine and causes grey mould (Nair & Hill, 1992). However, studies with *B. cinerea* on various aspects, such as timing of fungicide application, biological control, host resistance and disease prediction models, usually comprise investigations on bunches. The rationale for this is that the most prominent phase of the disease is on berries (Harvey, 1955; McClellan & Hewitt, 1973; Jarvis, 1980; Nair, 1985; Nair & Parker, 1985). Recent infection studies (Holz *et al.*, 1997, 1998; Coertze & Holz, 1999) revealed that latent pedicel infections are more important in Botrytis bunch rot, than latent infections of the style–end (McClellan & Hewitt, 1973; Nair & Parker, 1985) or cheek of berries (Nelson, 1956). Gütschow (2001) showed that *B. cinerea* infects and commonly occured in the structural bunch parts of immature bunches. These immature bunches carried more *B. cinerea* than the berry cheek, and the different parts, shoots, petioles, leaf blades and inflorescences, all carried very high *B. cinerea* inoculum levels. Rachises, laterals and pedicels were less resistant than the berry cheek, and mostly carried higher latent infection. This suggests that more emphasis should be placed on control of the disease on structural bunch parts, rather than on the berry.

Strategies to reduce Botrytis bunch rot rely heavily on fungicide sprays to prevent berry infections during the growing season. The target to which fungicides are applied, constantly changes, because the shape and form of the grape bunch varies as it grows and ripens (Thwaites, 2001). Penetration of fungicides into the tightening clusters may therefore become increasingly difficult and inner surfaces may remain inadequately protected against infection by the pathogen. Control by cultural, chemical and biological means can, however, only be achieved by reducing inoculum at the correct infection site and appropriate developmental stage (Gütschow, 2001).

INFECTION

Inoculum Dispersal

Botrytis cinerea exists in grapevine as sclerotia (Nair & Nadtotchei, 1987), conidia (Corbaz, 1972; Bulit & Verdu, 1973) and mycelia (Gessler & Jermini, 1985; Northover, 1987). Sclerotia of *B. cinerea* have been shown to be a source of primary inoculum for bunch rot of grapes (Nair & Nadtotchei, 1987), and studies on the structure and development of these sclerotia are necessary to understand the biology of the infection process and aid in the formulation of control measures. Sclerotia are generally considered to be the most important structures involved in the survival of *Botrytis* species (Coley–Smith, 1980). Studies done by Nair and Martin (1987) on the ultrastructure of the sclerotia of *B. cinerea* suggested that they are well adapted for relatively long periods of survival. Two features likely to facilitate survival were the deposition of melanin on the surface and the presence of inner cells that are equipped with electron-dense storage bodies. The sclerotia may be directly infective or a source of conidia that can infect grapevines (Jarvis, 1980).

The conidia of *Botrytis* species are usually considered to be short–lived propagative spores, but there is evidence that under certain conditions even these may have considerable powers of survival (Coley–Smith, 1980). Conidia are dispersed in air currents (Jarvis, 1962), in splashing water droplets (Jarvis, 1962), and by insects (Fermaud & Le Menn, 1989; Fermaud & Gaunt, 1995; Louis *et al.*, 1996). It has been postulated that, following air and water dispersal, infection by solitary conidia should play a prominent role in the epidemiology of *B. cinerea* on grapevine (Coertze & Holz, 1999; Coertze *et al.*, 2001).

The association of *B. cinerea* with the grape berry moth larvae, *Lobesia botrana* Denis & Schiffermeuller, suggests that this insect plays an important role in dissemination of the pathogen (Fermaud & Le Menn, 1989). *Thrips obscuratus* Crawford, the New Zealand flower thrip, was capable of carrying conidia of *B. cinerea* on its body (Fermaud & Gaunt, 1995). According to Louis *et al.* (1996), conidia of *B. cinerea* are carried externally on the cuticle of the "fruit fly", *Drosophila melanogaster* Meig, and may also be carried internally through the digestive tract. Conidia germinated in the insect crop and developed into mycelia. Under some conditions, development of microsclerotia, which are fungal survival structures, may occur, which the flies can carry their entire life. *Drosophila* flies, once infected,

therefore became a potential reservoir of the pathogen in three ways: conidia, mycelia and microsclerotia (Louis *et al.*, 1996).

Floral debris bearing mycelia are dispersed by wind and rain and provide a large saprophytically based inoculum adpressed to plant surfaces when wet (Jarvis, 1980).

Adhesion of conidia

Adhesion of fungal spores to plant surfaces is an important stage in the infection process and the epidemiology of plant diseases (Spotts & Holz, 1996). Adhesion of conidia of *B. cinerea* occurs at two stages (Doss *et al.*, 1993). Immediate adhesion, the first stage, occurs upon hydration of freshly deposited conidia. Immediate adhesion is characterised by relatively weak attachment forces and is the strongest with hydrophobic substrata. The second stage, which only occurs with viable conidia and is not influenced by the hydrophobic character of the substratum, indicates a delayed adhesion. Delayed germination occurs after viable conidia have been incubated for several hours under conditions that promote germination. This stage also involves secretion of an ensheathing film, referred to as the fungal sheath, that remains attached to the substratum upon physical removal of the germlings (Doss *et al.*, 1995). Extracellular mucilages are common on fungal germlings, including *B. cinerea* (McKeen, 1974; Jarvis, 1980). Spore tip mucilage (Hamer *et al.*, 1988), adhesive knobs (Dijksterhuis *et al.*, 1990) and mucilage covered appendages (Bird & McKay, 1987) may be involved in adhesion of ungerminated conidia from other species.

Germination

Germination of conidia and the development of germ tubes are stimulated by various factors like pollen (Chou & Preece, 1968), glucose and fructose (Kosuge & Hewitt, 1964) and aqueous extracts of the stigma and style (McClellan & Hewitt, 1973). Conidia germinates more extensively on wet berries, but germ tube elongation differs at different phenological stages (Coertze *et al.*, 2001). The conidia of *Botrytis cinerea* formed germ tubes with lengths up to 150µm before an appressorium was formed on Tokay grapes (Nelson, 1956). McKeen (1974) found on leaves of *Vicia fabae* L. that the turning down of the tip of the germ tube was the first indication that infection was about to occur. The tips of the germ tubes were held firmly against the cuticle of the leave by mucilage that spread some distance around the germ

tube. *Botrytis cinerea* forms a variety of penetration structures before penetration of the cuticle. The fungus forms protoappressoria, simple appressoria, multicellular lobate appressoria and infection cushions on the flower parts of plum and nectarine (Fourie & Holz, 1994).

Penetration

Different infection pathways have been described for *B. cinerea* on grape berries, namely stylar ends (McClellan & Hewitt, 1973; Nair & Parker, 1985), pedicels (Pezet & Pont, 1986; Holz *et al.*, 1997, 1998), natural openings (Pucheu-Planté & Mercier, 1983), wounds (Nair *et al.*, 1988), or by direct penetration of the cuticle (Nelson, 1956). According to McKeen (1974), a pore developed in the fungal wall in the centre of the contacting germ tube. The infection peg, covered by the plasmalemma, was pressed against the host cuticle, and the plasmalemma covered the infection peg as it moved through the cuticle (McKeen, 1974). Although McKeen (1974) reported that the infection pegs lack walls, Backhouse and Willets (1987) observed thin walls around the infection pegs that appeared to be different in structure and composition from hyphal walls. The germ tubes of *B. cinerea* usually penetrated undamaged onion leaves directly via the anticlinal cell (Clark & Lorbeer, 1976). A very thin penetration peg grew from the tip of the germ tube or from an appressorium. Nelson (1956) also noticed an infection peg coming from the underside of an appressorium that penetrated directly through the cuticle, after which it enlarged into a subcuticular and intercellular mycelium. Studies by Holz *et al.* (1998) on the behaviour of *B. cinerea* on the berry surface showed that the pathogen does not necessarily follow the infection pathway as described in literature. It seems as if two inoculum types are involved in berry infection, namely mycelia and conidia. The more important infection pathway is via the pedicel (fruit stem) and this infection pathway is symptomless. There are clear indications that resistance mechanisms operate in the pedicel and that latency is settled there. These mechanisms are highly effective and destroy a large proportion of the latent infections in the pedicel. However, these mechanisms do seem to subside as bunches develop and the pathogen can systemically grow along the vascular tissue out of the pedicel and into the berry. This type of inoculum therefore reaches the berry from inside and is not affected by the resistance mechanisms that prevent penetration of the berry skin (Holz *et al.*, 1998).

Penetration through stomata, lenticels or micro cracks

Müller-Thurgau (cited in Nair & Hill, 1992) observed in 1888 that infection of grapes by *B. cinerea* occurred through lenticels. According to Nelson (1951), lenticels, insect punctures and microscopic injuries were not essential courts of infection. Clark and Lorbeer (1976) noticed that conidia of *B. cinerea*, when inoculated in a nutrient broth, frequently penetrated stomata without forming appressoria. According to Verhoeff (1980), Bessis also found no proof for direct penetration of the berry cuticle, and concluded that the pathogen penetrates grape berries through minute cracks or openings in the cuticle. Isolation studies done by Coertze *et al.* (2001) revealed that berry cheeks were free from *B. cinerea* infections. Only 2% of berries bearing the receptacle part of the pedicel yielded the pathogen, which developed primarily on the receptacle, from where it colonised the berry.

Penetration through wounds

Early studies (Vanderwalle (cited in Nelson, 1951), and Du Plessis, 1937) reported that wounded grapes were more susceptible to *Botrytis* infection than unwounded grapes. According to Edlich *et al.* (1989), *B. cinerea* is predominantly a wound pathogen under field conditions. Gärtel (cited in Verhoeff, 1980), found that hail damage on grape berries led to quick infection by *B. cinerea*. Injuries of grape clusters, resulting from insect feeding or from the expansion of berries in tight clusters, might be important avenues for *B. cinerea* infections (Savage & Sall, 1983). Studies by Coertze *et al.* (2001) indicated that grape skins provided an effective barrier to penetration by solitary conidia. This confirms the decisive role of wounding in both symptom expression and the epidemiology of *B. cinerea* on grapevine. Wounds are regarded as major entry sites for the pathogen on grapes (Du Plessis, 1937; Hill *et al.*, 1981; Nair *et al.*, 1988; Coertze & Holz, 1999). Grapes can be wounded by insects, frost, hail, windblown sand, sunburn, or the rapid uptake of water leading to splitting (Jarvis, 1980; Savage & Sall, 1983).

Penetration through flower parts

Penetration through flower parts is considered a very important pathway through which *B. cinerea* gains entrance into fruit. McClellan & Hewitt (1973) and Nair & Parker (1985) found that the pathogen invaded the stigma and style–end of grape bunches during bloom. Nair (1985) isolated *B. cinerea* from apparently healthy and surface-sterilised flowers. Powelson (1960) showed that *B. cinerea* expanded from latent infections of floral parts into the receptacle and concluded that the calyx is the primary pathway for *B. cinerea* on strawberries. Studies by De Kock and Holz (1992) on pears showed that *B. cinerea* entered the fruit through the stamens and sepals and became latent in these tissues. Fourie and Holz (1994) found that infected floral parts of nectarines and plums did not remain attached to the young developing fruit. Therefore, floral parts of nectarines and plums did not serve as infection pathways to the fruit.

Latent infections

Studies on the epidemiology of bunch rot of grapes in the Hunter Valley, Australia (Nair, 1985; Nair & Parker, 1985) have shown that the disease may not only be based on *de novo* infection of split, mature grapes, but also on the development of latent infection established earlier in the flowers. Pezet and Pont (1986) defines latency as "infection of a host plant by a parasite without the development of visible symptoms during a certain period". Latency is an important aspect in disease because early asymptomatic infection results in rotting later in the season. These infections are important because they are difficult to quantify, difficult to control and they fulfil a largely unexplored part in the development of infection (Holz *et al.*, 1998). The recognition of latency places more emphasis on the timing of fungicide application to control the disease. Knowledge of such a dormancy period in the process of infection of a plant by a parasite is of primary importance in combating the disease to which it gives rise. In fact, during the latency period the parasite is protected inside the grape against contact fungicides. Studies done by De Kock and Holz (1992) has shown that *B. cinerea* can enter the flower receptacle or mesocarp tissue of immature pear fruit from stamens and sepals and become latent in these tissues. Similarly, *B. cinerea* can penetrate into flower parts or the tissues of the developing fruit of strawberry (Bristow *et al.*, 1986), grape (McClellan & Hewitt, 1973; Pezet & Pont, 1986), red raspberry (Dashwood & Fox, 1988), black current (McNicol & Williamson, 1989), almond and apricot (Ogawa & English, 1960) and apple

(Tronsmo & Raa, 1977) to establish latent infections. A pathogenic relationship is however, not established until the fruit ripens. Studies (Holz *et al.*, 1997, 1998; Gütschow, 2001) indicated that latent pedicel infections are more important in Botrytis bunch rot that latent infections of the style–end or cheek of berries.

Verhoeff (1980) suggested three possibilities to explain the transition from a quiescent to an active pathogenic relationship. Firstly, the immature fruit may contain a substance toxic to the fungus, that disappears at maturity. Secondly, the immature fruit does not contain the nutritive substances required by the fungus for its development. These substances appear at maturity. It is well known that the concentration of sugars in fruit increases as they mature and ripen. It has been reported that grape berries with a high sugar content are more susceptible to infection (Nelson, 1951; Stalder, 1953; Kosuge & Hewitt, 1964). Thirdly, the fungus may be unable to produce enzymes essential to its development; if, however, it is capable of producing them, these enzymes are deactivated in the immature fruit (Verhoeff, 1980). Nair and Parker (1985) and Nair *et al.*, (1995) suggested fungicide sprays at pre-bloom and at the start of fruit development to prevent the establishment of latent infections.

Bunch architecture

In addition to physically confining tissues to maintain a firm compact form, the cuticular membrane serves to reduce water loss due to transpiration, contributes to controlled gaseous exchange, restricts the reaching of essential compounds and nutrients, protects the plant from injuries (e.g. physical abrasion, frost and harmful radiation) (Martin & Juniper, 1970) and provides the main constitutive (i.e. performed) defense mechanism against pathogens such as *B. cinerea* (Heath, 1984; Marois *et al.*, 1986). The epicuticular wax layer of a mature grape berry is semi-crystalline to crystalline in structure (Possingham *et al.* 1967). It influences the retention of pesticides, the wettability of the berry surface and the adhesive ability of plant pathogens (Baker, 1982; Hallam, 1982; Gay & Pearce, 1984; Heath, 1984; Nicholson, 1984). The phenomenon of tight, compressed clusters in some grape varieties is associated with the development of severe Botrytis bunch rot. Two main reasons most often cited to explain this relationship are that the tighter clusters do not dry as fast as loose clusters after rain or heavy dew, and that the tight clusters often cause berries to rupture (Marois *et al.*, 1986). According to Nair and Parker (1985), grape cultivars with bunches that were looser, berries less compressed and better aerated, had lower infection. Compression between berries in the

bunch caused splitting and partial severance of the grape from its pedicel, thus making it more susceptible to infection by *B. cinerea* (Jarvis, 1980). Marois *et al.* (1986) determined the effect of berry contact within the cluster on the susceptibility of the berries to infection by *B. cinerea*. They found that the contact surface had a higher proportion of infection. Berries that occured in tight clusters were more susceptible to *B. cinerea* than those in loose clusters (Savage & Sall, 1983; Marois *et al.*, 1986; Gubler *et al.*, 1987; Vail & Marois, 1991). Nair and Parker (1985) found that infection first appeared on berries that were inside a bunch and later spread outwards to the outer berries. Studies done by De Kock and Holz (1991) revealed that post harvest decay is largely due to infection by inoculum present in bunches. This confirmed the findings of Northover (1987) that infected dead floral parts remained in the clusters, and most probably served as foci for the increase in cluster infection observed during berry ripening. Cluster architecture influences the length of time that the cluster retains water (Vail & Marois, 1991). It was found that tight clusters dried at a slower rate than loose clusters. Infection of grape berries by *B. cinerea* is very common in cultivars with dense canopies (Savage & Sall, 1983; Gubler *et al.*, 1987). Trellis types therefore play an important role in Botrytis diseases. The "two-wire vertical" trellis was associated with lower rot incidence than the "crossarm" trellised blocks (Savage & Sall, 1983, 1984). According to Northover (1987), fungicide applications between flowering and the stage of pea-sized berries probably serve to prevent colonization of floral debris by *B. cinerea*. Later applications serve to protect the ripening berries and pedicels. As the berries increase in size, penetration of the fungicide into the tightening clusters becomes increasingly difficult and the inner surfaces remain inadequately protected.

CHEMICAL CONTROL

Botrytis cinerea is a facultative parasite which parasitises over 200 host species (Jarvis, 1980). Due to its ubiquitous nature, large host-range and saprophytic abilities, most producers rely solely on the use of chemicals to control this pathogen, both during the pre- and post harvest stages. The resistance of this pathogen to fungicides in several vineyards necessitates the development of new fungicides and fungicide programs for the principal cultivars.

Fungicides

Benzimidazole fungicides were extensively used to control *B. cinerea* in the field (Delp & Klopping, 1968; Dekker, 1976). They lost part of their importance during the last decade on most crops due to the appearance and persistence of resistant strains (Smith, 1988), and to toxicological problems (Gullino *et al.*, 1992, and have limited use on grapevine and vegetable crops (Gullino & Garibaldi, 1982). After only a few years of widespread and intensive use, field resistance to benzimidazoles was reported in *B. cinerea* populations (Smith, 1988). In the mid 1970's, dicarboximide fungicides were developed that were particularly effective against *B. cinerea* (Lorenz, 1988). Dicarboximides were therefore timely successors to benzimidazoles for the chemical control of this economically important pathogen. However, due to the extensive usage of dicarboximide fungicides, *B. cinerea* developed resistance to these fungicides in different populations worldwide (Lorbeer & Vincelli, 1990; Moorman & Lease, 1995; Fourie, 1996; Fourie & Holz, 1998).

Dicarboximides. The 3,5-dichlorophenyl-*N*-cyclic imide ('dicarboximide') fungicides iprodione, procymidone and vinclozolin, introduced in the mid 1970's, are primarily used to control the grey mould fungus, *B. cinerea* (Pommer & Lorenz, 1982). These chemicals are mainly protectant fungicides, but they also possess some ability to penetrate plant tissues, as well as a slight curative action (Lorenz, 1988). Dicarboximides are generally used on a wide range of crops and are effective against fungi of the genera *Botrytis, Sclerotinia* Fuckel, *Monilia* Bonorden, *Sclerotium* Tode and *Phoma* Fr. (Pommer & Lorenz, 1982, 1987).

All dicarboximides inhibit mycelial growth and conidial germination of *B. cinerea* (Eichhorn & Lorenz, 1978; Pappas & Fischer, 1979; Pommer & Lorenz, 1982, 1987; Edlich & Lyr, 1987). The visible effect of these compounds on hyphal cells and germ tubes is often observed in swelling and bursting, followed by extrusion of cytoplasm, that suggests the direct or indirect effect on cell wall synthesis (Eichhorn & Lorenz, 1978; Albert, 1981) and cell wall or membrane integrity (Davis & Dennis, 1981). Stunting and swelling of germ tubes and changes in hyphal morphology have also been observed (Davis & Dennis, 1981; Pommer & Lorenz, 1982, 1987; Edlich & Lyr, 1987; Sisler, 1988). Georgopoulos (1977) observed that dicarboximides also caused mitotic instability in *Aspergillus nidulans*, thereby disrupting mitotic division. A biochemical assay to determine the mode of action of dicarboximides was done by Pappas and Fischer (1979). No general primary mechanism of action could be found.

They hypothesised that the chemical moiety may facilitate penetration into the fungal cell, but the ultimate toxic action of the various compounds may be different. DNA synthesis was inhibited by iprodione, and all the compounds inhibited chitin metabolism. Gullino and Sisler (1986) suggested that the observed antagonism between iprodione and mixed function oxidase inhibitors is an indication that the fungitoxicity of iprodione relies on an activation catalysed by a cytochrome P-450 mixed function oxidase. Further work is required to elucidate the primary targets of the dicarboximide compounds.

Due to the extensive usage of dicarboximide fungicides, *B. cinerea* developed resistance to these fungicides in different populations worldwide (Fourie, 1996; Fourie & Holz, 1998; Lorbeer & Vincelli, 1990; Moorman & Lease, 1995). Accordingly, the wisest use of the dicarboximide fungicides would be in combination with other protectant fungicides to delay build–up of such resistance (Lorbeer & Vincelli, 1990).

Cyanopyrrole. ***Fludioxonil.*** A new compound introduced recently against *Botrytis* and several seedborne cereal pathogens is fludioxonil (CGA 173506) from the novel fungicide class of the phenylpyrroles (Gehmann *et al.*, 1990). Fludioxonil can be used as a foliar fungicide against pathogenic genera, such as *Botrytis* and *Monilinia* Honey (Lyr, 1995). It is active at low rates for seed treatment against *Fusarium* Link, *Septoria* Fr., *Tilletia* Tul. & C.Tul. and *Helminthosporium* Link, or in rice against *Gibberella* Sacc. (Lyr, 1995). No cross resistance between fludioxonil and benzimidazoles or guanidines was found (Gehmann *et al.*, 1990), however, Faretra and Pollastro (1992) obtained resistant mutants of *B. cinerea* in the laboratory with various degrees of resistance to fludioxonil. Fludioxonil blocks a protein-kinase, that catalyses phosphorylation of a regulatory enzyme of glycerol synthesis (Anonymous, 1999).

Anilinopyrimidines. ***Pyrimethanil.*** Pyrimethanil (*N*-(4,6-dimethylpyrimidin-2-yl) aniline), an active ingredient from the anilinopyrimidine chemical family, has recently been introduced for the control of *B. cinerea* in grapevines and other susceptible crops (Neumann *et al.*, 1992; Milling & Richardson, 1995; Jalil *et al.*, 1998). It is characterised by a novel mode of action, namely inhibition of enzyme secretion (Miura *et al.*, 1994; Milling & Richardson, 1995), an action at the level of amino acid biosynthesis (LeRoux, 1994; Masner *et al.*, 1994). Laboratory studies have established that pyrimethanil is highly active against all strains of *B. cinerea*, including those resistant to other botryticides, such as benzimidazoles,

dicarboximides and *N*-phenylcarbamates (LeRoux & Montcomble, 1993; LeRoux & Lagouarde, 1994). It is most active in media where the fungus has to utilise extracellular enzymes to mobilise the nutrients it requires for growth (Milling & Richardson, 1995). Studies done by Daniels and Lucas (1995) on the action of pyrimethanil against *B. fabae* Sardina on broad bean leaves, suggested that pyrimethanil had little reproducible activity on pre-penetration stages of development in *B. fabae*, at least at expected field rates. Instead, the major effect of the compound appeared to be interference with a later stage in the host-pathogen interaction, preventing lesion expansion. Grinstein *et al.* (1997) studied the effect of deposit and cover density of fungicides on the development of Botrytis blight on roses. It was observed for pyrimethanil that the drop size had practically no effect on the control of disease, whereas the concentration of the fungicide did affect the control efficacy. They concluded that the vapour action of pyrimethanil can produce effective control of Botrytis blight of rose cut flowers caused by *B. cinerea* (Grinstein *et al.*, 1997). Stensvand (1997) found, when evaluating two new fungicides and a biocontrol agent against grey mould in strawberries, that the percentage of berries were significantly lower with the pyrimethanil (150 ml) and fenhexamid (Teldor®) treatments than the other treatments. He concluded that pyrimethanil and fenhexamid may be future alternatives for iprodione and vinclozolin. In order to maintain full potential of pyrimethanil in the long term, Fabreges and Birchmore (1998) advised that it is continued to be used in the framework of the manufacturer's recommendations, only applying it as one treatment out of three per season, alternating with other fungicides from different chemical families. The novel mode of action of pyrimethanil contrasts with the known activity of other currently available fungicides used against *Botrytis* species, and indicates that pyrimethanil should prove suitable for use in alternating spray schemes for resistance management.

Cyprodinil. Cyprodinil (CGA 219417) (*N*-(4-cyclopropyl-6-methyl-pyrimidin-lyl) aniline) is a new member of the anilinopyrimidine fungicides (Lyr, 1995). Cyprodinil has a particularly wide spectrum of activity with powdery mildews, eyespot of cereals, apple scab and *Botrytis* being the major targets (Heye *et al.*, 1994). Besides *Botrytis* Fr. and *Venturia* Sacc., *Pseudocercosporella* Deighton, *Erysiphe* Hedw. f. ex DC., *Septoria* Fr. and *Rhynchosporium* Heinsen ex Frank spp. can also be controlled (Lyr, 1995). The mechanism of action is an inhibition of aminoacid synthesis. Cyprodinil inhibits the biosynthesis of methionine and acts on secretion of hydrolytic enzymes (Kühl and Raum, cited in Lyr, 1995).

Hydroxyanilides. ***Fenhexamid.*** Fenhexamid (*N*-(2,3-dichloro-4-hydroxyphenyl)-1-methyl-cyclohexanecarboximide) is a new foliar fungicide, with protective action, from the new chemical class of the hydroxyanilides. It shows activity against *B. cinerea, Monilinia* spp. and *Sclerotinia sclerotiorum* (Lib.) De Bary in grapes, berries, stonefruits, citrus, vegetables and ornamentals, and has excellent plant compatibility (Rosslenbroich *et al.*, 1998). It has short pre-harvest intervals, excellent lasting activity and gives good protection for stored soft fruits (Rosslenbroich *et al.*, 1998). Fenhexamid has a new mode of action and shows no cross-resistance to other known botryticides. It inhibits fungal germ tube elongation and mycelium growth. Studies done by Adam and Birch (1998) on fenhexamid 50 WG for the control of *B. cinerea* on soft, cane and bush fruit crops in Great Britain, demonstrated good control of *B. cinerea* on all crops. A rate of 750 g a.i. fenhexamid/ha, applied as a programme of treatments from early flowering, demonstrated good control of *B. cinerea* on all crops, and was at least as effective as the commercial standard treatment. Fenhexamid programmes also gave good reduction in post harvest and storage rots in all 3 crops (Adam & Birch, 1998).

TIMING OF FUNGICIDE APPLICATIONS

On grapevine, studies with *B. cinerea* on various aspects such as timing of fungicide application, biological control, host resistance and disease prediction models, usually comprised investigations on mature berries. In other words, all basic research done on disease control, epidemiological studies and disease forecasting, used the berry as medium and criterion. Incidence of Botrytis bunch rot and disease severity is usually estimated by using rating scales (Kremer & Unterstenhüfer, 1967; Pearson & Riegel, 1983; De Kock & Holz, 1994) to assess the efficacy of fungicide applications. These studies have resulted in the recommendation of various window periods for the control of *B. cinerea*: fungicide applications at pre-bloom and a second application during bloom (Nair *et al.*, 1987), sprays when berries were pea-size and again at bunch closure to early ripening (Northover, 1987), only two late season applications during véraison and before harvest (Pearson & Riegel, 1983; De Kock & Holz, 1991,1994), or simply just reducing the number of treatments (Wang & Coley-Smith, 1986). Fungicide applications between flowering and the stage of pea-size berries probably serve to prevent colonization of floral debris by *B. cinerea*, whereas later applications serve to protect the ripening berries and pedicels from infection by mycelial spread from infected floral debris remaining within the clusters (Northover, 1987). The target

to which fungicides are applied constantly changes, because the shape and form of the grape bunch varies as it grows and ripens (Thwaites, 2001). Penetration of fungicide into the tightening clusters may therefore become increasingly difficult and the inner surfaces may remain inadequately protected against infection by the pathogen.

Control by cultural, chemical and biological means can, however, only be achieved by reducing the inoculum types at the correct infection site and appropriate developmental stage. It is therefore important to consider the efficacy of fungicides in terms of control at different morphological parts and infection sites. Knowledge of the infection of *B. cinerea* at various sites in grapevine will result in correct identification of window periods and thus correct timing of fungicide application.

INTEGRATION OF DISEASE CONTROL STRATEGIES

The risk of resistance build–up is much higher in crops that rely heavily on chemical control, such as grapes, and consequently, other measures such as biological, cultural and genetic practices should be exploited optimally.

Biological control agents such as fungi, bacteria and yeasts show a significant degree of antagonism to *B. cinerea* (Dubos, 1992; Edwards & Seddon, 1992; Gullino *et al.*, 1992; Köhl *et al.*, 1992; Leifert *et al.*, 1992; Malathrakis & Kritsotaki, 1992) and can be used in integrated control programs with fungicides (Elad & Zimand, 1992; Malathrakis & Klironomou, 1992). Cultural practices are the most important factor in integrated control that is often overlooked. Measures such as the reduction of nitrogen supplies (Chambers *et al.*, 1993), removal of excessive foliage to improve air circulation, spray penetration, and to reduce relative humidity, minimising insect injury to bunches, and effective treatment of grape-worms, downy mildew (*Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni) and powdery mildew (*Uncinula necator* (Schwein.) Burrill) control by using compounds that have a secondary effect on *B. cinerea* (LeRoux & Clerjeau, 1985; Löcher, 1988), will lower the disease incidence and severity. Consequently, fewer fungicide applications will be needed, and resistance development will decline.

By integrating all these measures, it is most likely that the development of resistance will be reduced in such a way that all botryticides could still be used in future. A broader

fungicide arsenal is of the utmost importance (Staub & Sozzi, 1984; Creemers, 1992), but with the pressure from environment-friendly societies, the emphasis should be placed on cultural practices.

CONCLUSION

Many factors predispose grapes to infection by *B. cinerea*. These factors include nutrients (Clark & Lorbeer, 1976; Blakeman, 1980; Verhoeff, 1980), wounding (Ciccarone, 1959; Jarvis, 1980), grape bunch architecture (Savage & Sall, 1983; Nair & Parker, 1985; Vail & Marois, 1991), packaging (Van der Sman *et al.*, 1996), crop fertilisers (Jarvis, 1980), atmosperic pollutants (Manning *et al.*, 1969) and many more. Environmental factors such as temperature (Yunis, 1990), relative humidity and wind speed can affect the development of *Botrytis cinerea* on the surfaces of inoculated grape berries (Thomas *et al.*, 1988). Preformed defense systems (Hill, 1985) and active defense mechanisms (Langcake, 1981; Hoos & Blaich, 1988) also play an important role in the resistance of grapevine to infection by *B. cinerea.*

Although much work has been carried out on the timing of fungicide applications on grapevine, there is a definite potential for increased control of *B. cinerea* by reducing inoculum types at the correct infection site and appropriate developmental stage. It has been postulated that, following air and water dispersal, infection by solitary conidia could play a prominent role in the epidemiology of *B. cinerea* on grapevine (Coertze *et al.*, 2001). This study will therefore focus on infection and fungicide efficacy at specific sites in grape bunches inoculated with dry, airborne *B. cinerea* conidia at all stages of development.

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2. INFECTION BY DRY, AIRBORNE *BOTRYTIS CINEREA* CONIDIA AND FUNGICIDE EFFICACY ON LEAF BLADES, PETIOLES, INFLORESCENSES AND NODES ON GRAPE VINELETS

ABSTRACT

Infection and fungicide efficacy was determined on shoots of grape vinelets inoculated with dry, airborne conidia of *Botrytis cinerea*. Vinelets (table grape cultivar Dauphine, wine grape cultivar Merlot), prepared from cuttings, were sprayed in a spray chamber at the recommended dosages with iprodione, pyrimethanil, cyprodinil/fludioxonil and fenhexamid, or left unsprayed. After 24 h the vinelets were dusted with dry conidia in a settling tower and incubated for 24 h at high relative humidity (±93%). Infection and fungicide efficacy was determined by observing intact vinelets for symptom expression, and by estimating the amount of *B. cinerea* at various sites on the vinelets with isolation studies. No symptoms of *B. cinerea* decay developed on sprayed and unsprayed vinelets that were kept in dry chambers during the 2 wk observation period. The pathogen however developed from the isolated parts. The isolation and incubation studies showed that the different fungicides were highly and nearly equally efficient in reducing superficial *B. cinerea* inoculum and latent infection. In the case of leaf blades, which showed a high amount of *B. cinerea* on unsprayed vinelets under two sterility regimes, decay was significantly reduced by each fungicide on both cultivars. This was not the case for the other parts, which yielded *B. cinerea* at low incidences under the two sterility regimes. The study showed that the fungicides used in this study, if applied properly to shoots at the prebloom stage, should effectively reduce *B. cinerea* infection in blades and petioles of leaves, and prevent the infection of nodes, internodes and inflorescences.

INTRODUCTION

Botrytis cinerea Pers.: Fr., a pathogen of grapevine (*Vitis vinifera* L.), can infect most of the plant's organs. Studies with *B. cinerea* on various aspects such as timing of fungicide application, biological control, host resistance and disease prediction models, however, usually comprise investigations on bunches. The rationale for this is that the most prominent

phase of the disease is found on berries (Harvey, 1955; McClellan & Hewitt, 1973; Jarvis, 1980; Nair, 1985; Nair & Parker, 1985). Incidence of disease severity is usually estimated by using rating scales on mature berries (Kremer & Unterstenhüfer, 1967; Pearson & Riegel, 1983; De Kock & Holz, 1991, 1994). These studies have resulted in the recommendation of four window periods for the control of *B. cinerea* in bunches (Pearson & Riegel, 1983; Wang & Coley-Smith, 1986; Nair *et al.*, 1987; Northover, 1987; De Kock & Holz, 1991,1994; LeRoux, 1995).

A recent investigation (Gütschow, 2001) on grapevine showed that the amount of *B. cinerea* at different sites on leaves and bunches may be higher during early season than generally assumed. Based on the occurrence of natural *B. cinerea* infection and disease expression of leafblades, petioles, rachises, laterals, pedicels and berries, the various sites were at all growth stages classified as resistant to moderately resistant to disease expression. However, at pea size and bunch closure, in spite of its resistance, nearly all the sites carried high to very high amounts of *B. cinerea*. In nearly all sites, amounts of the pathogen were lower at véraison and harvest. These findings suggest that control of *B. cinerea* infection by cultural, chemical and biological means can only be achieved by reducing inoculum at the correct infection site and appropriate developmental stage. It is therefore important to consider the efficacy of fungicides on grapevine in terms of control at different morphological parts and infection sites. Little information is available on fungicide efficacy against dry, airborne *B. cinerea* conidia on different parts of grapevine. The aim of this study was to determine infection and fungicide efficacy at specific sites on grape vinelets inoculated with dry, airborne conidia of *B. cinerea*. It has been postulated that, following air and water dispersal, infection by solitary conidia should play a prominent role in the epidemiology of *B. cinerea* on grapevine (Coertze & Holz, 1999; Coertze *et al.*, 2001).

MATERIALS AND METHODS

Grapevine material. Cuttings obtained during July to August from two vineyards (table grape cultivar Dauphine; wine grape cultivar Merlot) were kept overnight in a captab (500 WP) solution and stored at 4°C in moist perlite in plastic bags. Before an experiment, cuttings were removed from the bags and placed in warm water (50°C) for 30 minutes (Goussard & Orffer, 1979). Each cutting was cut into 5-6 cm lengths, with one dormant eye and inserted in holes in foamalite strips. The foamalite strips with cuttings were placed in

stainless steel trays (53 x 28 x 2 cm) filled with tap water and kept at 25°C in a growth room at high relative humidity (85% RH) to initiate budbreak. Approximately 2 wk after budding had commenced, the trays with vinelets were divided into two groups. Vinelets of one group were left unsprayed, those of the other group were sprayed with fungicides. Vinelets of both groups were used in the infection studies.

Fungicide treatment. The trays with vinelets were placed in a spray chamber and sprayed at recommended dosages (Nel *et al.*, 1999) with iprodione (Rovral Flo 255 SC, Aventis), fenhexamid (Teldor 500 SC, Bayer), cyprodinil/fludioxonil (Switch 62.5 WG, Syngenta) or pyrimethanil (Scala 40 SC, Aventis). Application was conducted through a window in the spray chamber, that consisted of a steel framework (800 x 1410 x 660 mm [height x length x width]) covered with plastic. The fungicides were applied to runoff with a gravity feed mist spray gun (ITW DEVILBISS Spray Equipment Products) used at 2 bar. To ensure maximum coverage the spray mist was allowed to settle for 5 min on the vinelets, after which the vinelets were removed from the chamber and air-dried. After each spray, the chamber was well ventilated and cleaned before the next application. Following fungicide treatment, the vinelets were kept for 24 h at 22°C before inoculation.

Inoculation. A virulent isolate of *B. cinerea* that was obtained from a naturally infected grape berry was maintained on potato dextrose agar (PDA; 12 g Biolab agar, 200 g potatoes, 20 g sucrose, 1000 ml H_2O) at 5°C. For the preparation of inoculum, the isolate was first grown on canned apricot halves. Conidiophores from the colonised fruit were transferred to PDA in Petri dishes and incubated at 22°C under a diurnal regime (12 h near ultraviolet light; 12 h dark light). Dry conidia were harvested with a suction-type collector from 14 day old cultures and stored at 5°C until use (1 – 16 weeks). Storage time did not affect germination; the dry conidia could therefore be used in all experiments (Spotts & Holz, 1996). For inoculations, 3 mg dry conidia were dispersed by air pressure into the top of an inoculation tower (Plexiglass, 3 x 1 x 1 m [height x depth x width]) according to the method of Salinas *et al.* (1989) and allowed to settle onto the vinelets that were positioned in the foamalite strips in two trays. Petri dishes with water agar (WA; 12 g Biolab agar, 1000 ml H_2O) and PDA were placed on the floor of the settling tower at each inoculation and percentage germination was determined after 6 h incubation at 22°C (100 conidia per Petri dish, three replicates). Germination penetration varied between 92 and 99% at 6 h post inoculation. Following inoculation, the trays were placed in 12 ethanol-disinfected perspex (Cape Plastics, Cape

Town, South Africa) chambers (60 x 30 x 60 cm) lined with a sheet of chromatography paper with the base resting in deionised water to establish high relative humidity (≥93% RH). Each chamber was considered as a replicate and contained three trays with 20 vinelets per tray. The chambers were incubated for 24 h at 22°C with a 12 h photoperiod daily. These conditions provided circumstances commonly encountered in nature by the pathogen on grapevine surfaces, namely dry conidia on dry surfaces under high relative humidity. Studies (Gütschow, 2001) with dry conidia of *B. cinerea* on grape vinelets under similar conditions showed that germination and surface colonisation reached a maximum during this period.

Assessment of *B. cinerea*. Following incubation, the fungicide sprayed and unsprayed vinelets were divided in three groups respectively. Vinelets of the one group were surface sterilised in 70% ethanol for 5 s, those of the other two groups were left unsterile. Vinelets of one unsterile group (one vinelet per replicate, per treatment) were placed in dry chambers, kept for 14 days at 22°C with a 12 h photoperiod and monitored for symptom expression and the development of *B. cinerea* at different sites, namely the leaf blades, leaf petioles, shoots and inflorescenses. Vinelets of the other unsterile group, and the sterile group, were used for isolation. From each of these vinelets, 10 leaf blades, 10 leaf petioles, four shoots (approximately 20 mm each) and 10 inflorescenses were removed. Five each of the leaf blades, leaf petioles, inflorescences and two each of the shoots, were placed in Petri dishes on Kerssies' *B. cinerea* selective medium (Kerssies, 1990), and five on a water agar supplemented with paraquat (Grindrat & Pezet, 1994). The plates were incubated at 22°C under diurnal light and the segments were monitored daily for symptom expression and the development of *B. cinerea*. The presence of *B. cinerea* was recorded on the unwounded area of the different tissues only. Infection unequivocally associated with the cut-end of tissues were not recorded. The different treatments provided conditions that facilitated the development of *B. cinerea* by conidia on the surface of the vinelets, or by latent mycelia in the tissue, during the period of incubation. Previous studies (Coertze & Holz, 1999; Coertze *et al.*, 2001; Gütschow, 2001; Volkmann, 2001) with grape bunch tissue on Kerssies' medium showed that no superficial mycelial growth developed on the segments during the first 5 days of incubation. Hyphal growth usually occurred from cells underlying the cuticle into the medium after 5 days, which indicated direct penetration by conidia on the surface, or the development of latent mycelia from the host tissue during the incubation period. On segments from unsterile shoot parts, disease expression was therefore the result of infection by surface inoculum and the development of latent mycelia in host tissue. Decay incidences

on segments from a specific site therefore gave an indication of infection at that site as influenced by the amount of surface conidia and latent mycelia. Surface sterilization completely eliminated *B. cinerea* from the shoot surface (Sarig *et al.*, 1996; Coertze & Holz, 1999; Coertze *et al.*, 2001) and prevented infection by surface inoculum. Development of the pathogen from surface-sterilised shoot parts therefore gave an indication of infection at a specific site as influenced by latent mycelia. Paraquat terminates host resistance in the cells of the cuticular membrane without damaging host tissue, and thus facilitates the development of latent mycelia (Baur *et al.*, 1969; Cerkauskas & Sinclair, 1980; Pscheidt & Pearson, 1989; Grindrat & Pezet, 1994). After 9 days the number of segments yielding sporulating *B. cinerea* colonies were recorded, and the numbers used to quantify the amount of *B. cinerea* occurring at the various sites on the shoots.

Statistical analysis. The experimental design was a split plot repeated in 4 blocks. The main plot treatments were cultivar, medium and fungicide treatment. The sub-plot treatment was surface-sterilised and not sterilised. Statistical computations were performed using SAS (Statistical Analysis System, 1990). The data of the experiment, which was repeated, was subjected to analyses of normality of residuals ($P>0.05$ = normality) using the Shapiro and Wilk test for normality (Shapiro & Wilk, 1965). The data was examined further by using the analysis of variance (ANOVA) and the treatment means were compared using the Student's *t* LSD ($P = 0.05$) (Snedecor & Cochran, 1980).

RESULTS

Infection and fungicide efficacy on parts of vinelets kept intact. No symptoms of *B. cinerea* decay developed at any of the different sites on sprayed and unsprayed vinelets that were kept in dry chambers during the 2 wk observation period.

Infection and fungicide efficacy on parts used for isolation. The development of *B. cinerea* in the morphological parts followed a similar, constant pattern in both cultivars. Lesions were observed after 3 days on leaf blades and mostly developed from the area alongside the veins, and from the leaf basis. On the petioles, shoots and inflorescences, lesions usually were noted after 5 days. On shoots, lesions developed first and more often from the nodes than the internodes. No significant differences in *B. cinerea* disease incidence were found between repeated experiments. Thus, data from each of the two complete

experiments were combined. In the comparison of data, no significant differences were found in disease incidence between cultivars, and in disease incidence between tissues incubated on the two media (Table 1). However, the ANOVA of data showed that the interaction of cultivar, fungicide treatment, medium, sterility regime and infection site had a highly significant effect ($P < 0.01$) on *B. cinerea* incidence. On the unsprayed vinelets for both cultivars, disease incidence as expressed on the two media, and the two sterility regimes, were significantly higher on leaf blades than on the petioles, shoots and inflorescences. Disease expression at the latter sites fluctuated on the two media and sterility regimes, but was consistently at a low level in the shoots. In both cultivars, surface sterilisation of unsprayed vinelets reduced *B. cinerea* disease incidence at the different sites. This treatment had the result that disease incidence of internodes, which expressed *B. cinerea* at low levels in the unsterile regime for both cultivars, were reduced to 6.3% on the paraquat medium, and to 0% on Kerssies medium. On leaf blades, which expressed *B. cinerea* at high levels in the unsterile regime, disease incidence on both media were still high (≥60%) after surface sterilisation. The isolation and incubation studies showed that the different fungicides were highly and nearly equally efficient in reducing *B. cinerea* infection. In the case of leaf blades, which showed high disease expression of untreated tissue under the two sterility regimes, decay was significantly reduced by each fungicide on both cultivars. This was not the case on petioles, shoots and inflorescences, which yielded *B. cinerea* at low incidences under the two sterility regimes. Furthermore, on vinelets sprayed with fungicides, *B. cinerea* sporadically developed on one of the media from leaf blades and petioles, but seldom in the case of shoots and infloresences.

DISCUSSION

Laboratory studies showed (Gütschow, 2001) that although blades of mature grape leaves from commercial vineyards do not develop grey mould, they normally carried high levels of latent natural *B. cinerea* infection. It was futhermore showed (Gütschow, 2001) that young leaves on grape vinelets and older leaves from vineyard shoots remained asymptomatic after inoculation with dry, airborne *B. cinerea*. However, isolations made from these leaves indicated that they were highly susceptible and susceptible to infection, respectively. In this study, fungicides were applied in a spray chamber and inoculation conducted in a spore settling tower. These systems ensured proper fungicide coverage (G. Holz, unpublished data) and reduced sporadic occurrence of *B. cinerea* (Coertze & Holz, 1999; Coertze *et al.*, 2001),

thereby allowing uniform evaluation of fungicide efficacy at the different sites on the vinelets. Although the intact vinelets remained asymptomatic, the isolation studies confirmed that solitary conidia readily penetrated leaf tissue and that latent infection was established at very high levels in leaf blades. Latent infection was low in petioles, shoots and inflorescenses. The fungicides, that belonged to different chemical classes, all effectively reduced *B. cinerea* infection in leaves and petioles, and prevented shoot infection.

The finding that grape leaf blades carry high levels of latent natural *B. cinerea* suggest that leaf infection is an important primary infection event, and plays an important role in the epidemiology of the disease on grapevine. The role of latent *B. cinerea* mycelia in leaves in the epidemiology of gray mould on perennial strawberries is well established (Braun & Sutton, 1987; Sutton, 1998). Young leaves are highly susceptible to infections, that become latent. As the leaves senesce and die, the fungus colonises the tissues and sporulates (Braun & Sutton, 1987, 1988). Conidia formed on dying and necrotic leaves are the principal source of inoculum for Botrytis fruit rot epidemics. In a comparison of sanitation and fungicides for management of Botrytis fruit rot of strawberries, Merteley *et al.* (2000), showed that leaf sanitation (removal of senescent and necrotic leaves) reduced Botrytis fruit rot incidence compared to untreated controls. In grapevine, sclerotia are a source of conidia that result in primary infection of young tissue (Nair & Nadtotchei, 1987). Thomas (1983) showed that the bulk of sclerotia recovered from vineyard soils in the Western Cape province developed on vine leaves and shredded prunings. Sclerotia were formed on infected leaves that form a mat on the soil in autumn, or are covered under soil during winter (Thomas *et al.*, 1981). The optimum temperature for sclerotial germination followed by infection was between 20 and 25°C (Nair & Nadtotchei, 1987). Primary leaf infection prior to bloom is therefore likely when daily air temperature during spring reaches 20°C. Although leaf infection mostly remains asymptomatic, spots may develop and the leaf-infection sites can produce conidia abundantly during wet periods (Thomas, 1983; Nair & Hill, 1992) thereby contributing to the inoculum load that causes primary infection of nodes, internodes and inflorescences during the prebloom stage, of clusters during bloom and in the highly susceptible structural parts of grape bunches during pea size and bunch closure (Holz *et al.*, 1997, 1998). Most producers appear to be adhering to the recommendations (Pearson & Riegel, 1983; Wang & Coley-Smith, 1986; De Kock & Holz, 1991,1994; Nair *et al.*, 1987; Northover, 1987; LeRoux, 1995) that advocate four applications per season, namely at the end of flowering, at bunch closure, at véraison and three weeks before harvest. The finding during this study, that young leaves

are highly susceptible to infection, that inflorescences are infected by *B. cinerea* shortly after budburst, and the finding (Gütschow, 2001) that natural latent *B. cinerea* infection in leaves and bunches is the highest shortly after bloom, and lowest prior to harvest, suggest that the timing of fungicide application should be reconsidered. Thus, to effectively reduce *B. cinerea* in grapevine, three preventative applications are recommended to reduce primary infection events: (a) between budding and prebloom to counteract primary leaf infection; (b) during bloom, to prevent pimary infection of clusters; (c) and at bunch closure, to prevent infection of the inner bunch parts, especially for cultivars with tight bunches. The fungicides used in this study, if applied properly to shoots, should effectively reduce *B. cinerea* infection in blades and petioles of leaves, and prevent infection of the nodes, internodes and inflorescences.

It has been hypothesized that the development of bunch rot is most pronounced in cultivars that develop dense canopies and compact fruit clusters (Savage & Sall, 1983). This has resulted in the recommendation of various cultural practices that influence canopy management in order to reduce disease incidence and severity (Gubler *et al.*, 1987). The removal of basal leaves alters the microclimate within the grapevine canopy (English *et* al., 1989) and reduces the development of Botrytis bunch rot (Gubler *et al.*, 1987). Debris provides an initial nutrient source for *B. cinerea* and increases available inoculum for subsequent infections (Savage & Sall, 1984). This study showed that grapevine leaves carry high levels of latent *B. cinerea* inoculum and could serve as a reservoir for inoculum and subsequent infections when not removed during cultural practices. Leaves removed from vines during prebloom and during the early stages of bunch development should therefore be removed from the vineyard to reduce conidial and mycelial inoculum for infection of susceptible bunch parts.

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Table 1. Analysis of variance of data for the effect of cultivar, fungicide treatment, medium, sterility regime and infection site on the percentage parts isolated from vinelets that developed *Botrytis cinerea*

Source of variation	Df	MS	SL
Replicate	7	597.991	0.0034
Cultivar (C)	1	227.813	0.2695
Fungicide Treatment (FT)	4	45,194.648	0.0001
C x FT	4	79.961	0.7856
Medium (Med)	1	80.000	0.5122
C x Med	1	61.250	0.5663
FT x Med	4	1,932.930	0.0001
C x FT x Med	4	196.992	0.3773
Error(C x FT x Med)	133	185.256	
Sterility Regime (SR)	1	4,500.000	0.0001
C x SR	1	180.000	0.3661
FT x SR	4	1,912.695	0.0001
C x FT x SR	4	33.320	0.9618
Med x SR	1	0.313	0.9699
C x Med x SR	1	525.313	0.1237
FT x Med x SR	4	34.883	0.9585
C x FT x Med x SR	4	22.383	0.9816
Error(C x FT x Med x SR)	140	218.973	
Infection Site (IS)	3	17,989.479	0.0001
SR x IS	3	265.833	0.0731
C x IS	3	51.146	0.7188
C x SR x IS	3	187.500	0.1781
FT x IS	12	11,865.065	0.0001
FT x SR x IS	12	51.445	0.9424
C x FT x IS	12	103.294	0.5416
C x FT x SR x IS	12	82.487	0.7302
Med x IS	3	257.500	0.0805
Med x SR x IS	3	63.646	0.6432
C x Med x IS	3	248.750	0.0891
C x Med x SR x IS	3	93.646	0.4828
FT x Med x IS	12	341.680	0.0004
FT x Med x SR x IS	12	58.633	0.9069
C x FT x Med x IS	12	205.326	0.0443
C x FT x Med x SR x IS	12	290.716	0.0026
Error(C x FT x Med x SR x IS)	840	114.159	

Table 2. Means[v] of the effect of the interaction of cultivar x fungicide treatment x medium x sterility regime x morphological part on the percentage parts isolated from vinelets, sprayed with fungicides and inoculated with airborne *Botrytis cinerea* conidia, that yielded the pathogen on two different media[w]

	Dauphine								Merlot							
Fungicide	**Internode**		**Leaf Blade**		**Petiole**		**Inflorescence**		**Internode**		**Leaf Blade**		**Petiole**		**Inflorescence**	
Treatment	**K**	**P**	**K**	**P**	**K**	**P**	**K**	**P**	**K**	**P**	**K**	**P**	**K**	**P**	**K**	**P**
Control																
NS[x]	12.5 i-l	18.7 g-j	77.5 bc	95 a	30 f	47.5 e	27.5 fg	10 j-m	6.3 klm	12.5 i-l	67.5 cd	100 a	30 f	25 fgh	12.5 i-l	42.5 e
S[z]	0 m	6.3 klm	60 d	80 b	10 j-m	20 f-j	12.5 i-l	7.5 klm	0 m	6.3 klm	65 d	80 b	7.5 klm	22.5 f-I	10 j-m	12.5 i-l
Iprodione																
NS	0 m	0 m	0 m	0 m	12.5i-l	0 m	0 m	0 m	6.3 klm	0 m	5 klm	0 m	5 klm	2.5 l-m	0 m	0 m
S	0 m	0 m	0 m	2.5 m	0 m	0 m	0 m	0 m	0 m	0 m	0 m	0 m	20 f-j	0 m	5 klm	0 m
Pyrimethanil																
NS	0 m	0 m	5 klm	7.5klm	5 klm	2.5 lm	0 m	0 m	0 m	0 m	5 klm	10 j-m	0 m	5 klm	2.5 lm	5 klm
S	0 m	0 m	0 m	0 m	0 m	0 m	0 m	0 m	0 m	0 m	0 m	5 klm	0 m	2.5 lm	2.5 lm	0 m
Cyprodinil/fludioxonil																
NS	0 m	0 m	7.5 klm	0 m	10 j-m	0 m	0 m	0 m	0 m	0 m	10 j-m	5 klm	10 j-m	5 klm	0 m	0 m
S	0 m	0 m	2.5 lm	0 m	2.5 lm	0 m	0 m	0 m	0 m	0 m	5 klm	2.5 lm	12.5 i-l	0 m	0 m	0 m
Fenhexamid																
NS	0 m	0 m	15 h-k	0 m	5 klm	2.5 lm	0 m	0 m	0 m	0 m	7.5 klm	7.5 klm	7.5 klm	0 m	0 m	0 m
S	0 m	0 m	5 klm	2.5 lm	0 m	0 m	0 m	0 m	6.3 m	0 m	7.5 klm	2.5 lm	0 m	0 m	0 m	0 m

[v]Values in each column followed by the same letter are not significantly different according to the Student's *t*-test at P = 0.05.
[w]K = Kerssies medium, P = paraquat medium.
[x]NS = not sterile.
[z]S = surface sterilised.

3. INFECTION BY DRY, AIRBORNE *BOTRYTIS CINEREA* CONIDIA AND FUNGICIDE EFFICACY ON RACHISES, LATERALS, PEDICELS AND BERRIES IN GRAPE BUNCHES

ABSTRACT

Infection and fungicide efficacy was determined at specific sites in grape bunches (table grape cultivar Dauphine, wine grape cultivar Merlot) inoculated with dry, airborne conidia of *Botrytis cinerea.* The bunches were sprayed in a spray chamber at recommended dosages with iprodione, pyrimethanil, cyprodinil/fludioxonil or fenhexamid, or left unsprayed. After 24 h, the bunches were dusted with dry conidia in a settling tower and incubated for 24 h at high relative humidity (±93%). Infection and fungicide efficacy was determined by observing intact bunches for symptom expression, and by estimating the amount of *B. cinerea* at various sites in bunches with isolation studies. The study showed that dry, airborne conidia, and the fungicide sprays, penetrated loose and tight clustered bunches from bloom to harvest and evenly landed on the various bunch parts. At full bloom, the amount of *B. cinerea* in unsprayed bunches was high on the laterals and pedicels, but low on the embryos. Unsprayed intact bunches at full bloom were highly susceptible to *B. cinerea* and developed symptoms of grey mould. The fungicides inhibited symptom expression at full bloom, but could not prevent infection. Unsprayed bunches inoculated at the other stages remained asymptomatic. The amount of *B. cinerea* was generally high in the rachises and laterals at pea size and bunch closure stages, and in the pedicel end of the berries at harvest. Infection was constantly low in the berry cheek. The fungicides had a differential effect on infection at the various sites. In the case of rachises, the amount of *B. cinerea* was at each growth stage drastically reduced by each fungicide. In laterals, it was effectively reduced at pea size and bunch closure. However, at these two sites, significant differences in efficacy were found between the fungicides at stages when the amount of *B. cinerea* was high. This study showed that if these fungicides are applied properly to the bunches between budding and prebloom, during flowering, and at bunch closure, they should effectively prevent infection and symptom expression and thus the development of *B. cinerea* epiphytotics.

INTRODUCTION

Botrytis cinerea Pers.:Fr. attacks bunches, leaves, buds, and canes of grapevine (*Vitis vinifera* L.) and causes grey mould (Nair & Hill, 1992). Berries, on which the most prominent phase of the disease is found (Nair & Nadtotchei, 1987), are considered resistant to infection when immature, and susceptible when mature (Nelson, 1956; Hill *et al.*, 1981; Nair & Hill, 1992). In spite of this differential susceptibility, infection of flowers and berries may destroy immature fruit (McClellan & Hewitt, 1973; Nair & Parker, 1985). Flower infections by *B. cinerea* may also cause mid- and late-season bunch rot following a period of fungal latency (McClellan & Hewitt, 1973; Nair & Parker, 1985).

Knowledge about the pattern of natural occurrence of *B. cinerea* at different developmental stages in different sites in grape bunches is extremely important in planning control strategies. During flowering the pathogen can invade the stigma and then becomes latent in necrotic style tissue at the style-end of the berry. Grape clusters remain symptomless between the flowering period and the beginning of ripening, and a pathogenic relationship is generally established once the fruit ripens (McClellan & Hewitt, 1973; Nair & Parker, 1985). *Botrytis cinerea* also colonises the stamens during bloom and invades their base situated on the receptacle. From there it spreads via the vascular tissue into the pedicel-end of berries (Pezet & Pont, 1986). In addition, colonised senescent floral tissues and aborted berries can serve as conidial and mycelial inoculum (Gessler & Jermini, 1985; Hill, 1985; Nair & Nadtotchei, 1987; Northover, 1987) for late-season infections of sound berries. Studies with dry, airborne *B. cinerea* conidia showed that the skins of fresh ripe berries (Coertze & Holz, 1999), and berries at other growth stages (Coertze *et al.*, 2001), provided an effective barrier to penetration by this mode of infection. Consequently, few infections in grape berry cheeks are established by airborne conidia. Working with natural *B. cinerea* infection, Gütschow (2001) confirmed that berry cheeks are the most resistant sites in grape bunches, and that they carry the lowest level of latent infection. Rachises, laterals and pedicels are less resistant than the berry cheek, and mostly carry higher levels of latent infection. These findings imply that incipient infections can cause both mid- or late-season bunch rot following a period of fungal latency in the rachises, laterals or pedicels, and not in berry cheeks and style-ends.

Fungicides are currently used to control *B. cinerea* in grape bunches. The target to which fungicides are applied constantly changes, because the shape and form of the grape bunch varies as it grows and ripens (Thwaites, 2001). Penetration of fungicide into the tightening clusters may therefore become increasingly difficult and the inner surfaces may remain inadequately protected to infection by the pathogen. In this context, Nair and Parker (1985) found that infection first appeared on berries that were inside a bunch and later spread outwards to the outer berries. A recent study (Part 2) on fungicide efficacy on leaves, shoots and inflorescences of vinelets inoculated with dry, airborne conidia of *B. cinerea* showed that the fungicides tested were all highly and nearly equally efficient in reducing *B. cinerea* infection on leaves, which carried high latent infection levels in the unsprayed vinelets. Little information is available on fungicide efficacy against dry, airborne *B. cinerea* conidia on different parts of grape bunches. The aim of this study was to determine infection and fungicide efficacy at specific sites in grape bunches inoculated with dry, airborne conidia of *B. cinerea*. Sites in bunch parts were rachises, laterals and pedicels, and sites on berries were the pedicel-end, cheek and style-end. It has been postulated that, following air and water dispersal, infection by solitary conidia should play a prominent role in the epidemiology of *B. cinerea* on grapevine (Coertze *et al.*, 2001).

MATERIALS AND METHODS

Grape bunches. Two vineyards (table grape cultivar Dauphine, wine grape cultivar Merlot) with a history of low *B. cinerea* incidences were selected in the Paarl and Klapmuts region as source for experimental material. Material for infection and fungicide efficacy studies were selected at full bloom, pea size (3.5°Brix), bunch closure (4°Brix), véraison (Merlot 12.5° and Dauphine 8.3°Brix) and two weeks prior to harvest (Merlot 18.5° and Dauphine 15°Brix). At flowering stage, shoots with clusters were obtained from the vineyard, placed in flasks containing 20% sucrose solution to maintain turgidity, and transported to the laboratory. The shoots were cut back to approximately 20 cm, bearing three to five clusters and two to three leaves. The shoots were then inserted into sterile aluminium foil-wrapped "oases" (florist's sponge), soaked with a 20% sucrose solution to maintain turgidity. Due to the detrimental effect of ethanol, the inefficacy of surface-sterilisation and the fact that the clusters do not dry properly (G. Holz, unpublished data), surface sterilisation was not done at flowering stage. At the other stages, bunches were obtained, surface-sterilised (30 s in 70% ethanol, 2 min in 0.35% sodium hypochlorite, 30 s in 70% ethanol) and air-dried to prevent

natural infection by surface inocula (Sarig *et al.*, 1996; Coertze & Holz, 1999; Coertze *et al.*, 2001). Peduncles of bunches were inserted into sterile aluminium foil-wrapped "oases" (florist's sponge) soaked with a 20% sucrose solution to maintain turgidity. The oases with shoots or bunches were placed on sterile epoxy-coated steel mesh screens (53 x 28 x 2 cm). The screens were divided into two groups. Shoots and bunches of one group were sprayed with fungicides, those of the other group were left unsprayed. Both groups of shoots or bunches were used in the infection studies.

Fungicide treatment. The screens with shoots or bunches were placed in a spray chamber and sprayed at recommended dosages (Nel *et al.*, 1999) with iprodione (Rovral Flo 255 SC, Aventis), fenhexamid (Teldor 500 SC, Bayer), cyprodinil/fludioxonil (Switch 62.5 WG, Syngenta) or pyrimethanil (Scala 40 SC, Aventis). Application was conducted through a window in the spray chamber which consisted of a steel framework (800 x 1410 x 660 mm [height x length x width]) covered in strong plastic. The fungicides were applied to runoff with a gravity feed mist spray gun (ITW DEVILBISS Spray Equipment Products) used at 2 bar. To ensure maximum coverage the spray mist was allowed to settle for 5 min on the bunches, which were then removed from the chamber and air-dried. After each spray, the chamber was well ventilated and cleaned before the next application. Following fungicide treatment, the screens with shoots or bunches were kept for 24 h at 22°C in the laboratory (±56% RH) before inoculation.

Inoculation. A virulent isolate of *B. cinerea* that was obtained from a naturally infected grape berry was maintained on potato dextrose agar (PDA; 12 g Biolab agar, 200 g potatoes, 20 g sucrose, 1000 ml H_2O) at 5°C. For the preparation of inoculum, the isolate was first grown on canned apricot halves. Conidiophores from the colonised fruit were transferred to PDA in Petri dishes and incubated at 22°C under a diurnal regime (12 h near ultraviolet light; 12 h dark light). Dry conidia from 14 day old cultures, were harvested with a suction-type collector and stored at 5°C until use (1 to 16 weeks). Storage time did not affect germination, the dry conidia could therefore be used in all experiments (Spotts & Holz, 1996). For inoculations, 3 mg dry conidia were dispersed by air pressure into the top of an inoculation tower (Plexiglass, 3 x 1 x 1 m [height x depth x width]) according to the method of Salinas *et al.* (1989) and allowed to settle onto the shoots or bunches that were positioned on two screens. Petri dishes with water agar (WA; 12 g Biolab agar, 1000 ml H_2O) and PDA were placed on the floor of the settling tower at each inoculation and percentage germination

was determined after 6 h post inoculation (hpi) at 22°C (100 conidia per Petri dish, three replicates). Germination percentage varied between 92 and 99%. Following inoculation, the screens were placed in 12 ethanol-disinfected perspex chambers lined with a sheet of chromatography paper with the base resting in deionised water to establish high relative humidity (≥93% RH). Each chamber contained three screens containing eight bunches. Each chamber was considered as a replicate. The chambers were incubated for 24 h at 22°C with a 12-h photoperiod daily. These conditions provided circumstances commonly encountered in nature by the pathogen on grape bunches, namely dry conidia on dry bunch parts under high relative humidity. Studies (Coertze & Holz, 1999, Coertze *et al.*, 2001; Gütschow, 2001) with dry conidia of *B. cinerea* under similar conditions showed that germination, surface colonization and skin penetration reached a maximum during this period.

Assessment for *B. cinerea*. Following incubation at full bloom, the shoots were divided into two groups. Shoots of the one group were inserted into freshly prepared aluminium foil–wrapped oasis soaked with a 20% sucrose solution, placed in dry chambers and kept for 14 days at 22°C with a 12 h photoperiod. The clusters on the shoots were monitored daily for symptom expression of *B. cinerea*. Clusters on the other group of shoots were removed and used for isolation. They were carefully cut into short rachis sections bearing three to five groups of laterals with their pedicels and ovaries. From each cluster five rachis sections were isolated on Petri dishes containing Kerssies' *B. cinerea* selective medium (Kerssies, 1990), or on water agar medium supplemented with paraquat (Grindrat & Pezet, 1994). The plates were incubated at 22°C under diurnal light and the rachis sections were daily monitored for symptom expression and the development of *B. cinerea* on the rachis, laterals, pedicels and ovaries. After 11 days the number of rachis sections yielding sporulating *B. cinerea* colonies at any of the sites were recorded, and the numbers used to quantify the amount of *B. cinerea* occurring on the rachis sections.

At pea size, bunch closure, véraison and two weeks prior to harvest, the bunches were divided in three groups following incubation. Bunches of the one group were surface-sterilised in 70% ethanol for 5 s, those of the other two groups were left unsterile. Bunches of the one unsterile group (one bunch per replicate, per treatment) were inserted into freshly prepared sterile aluminium foil-wrapped oases soaked with a 20% sucrose solution, placed in dry chambers and kept for 14 days at 22°C with a 12 h photoperiod daily. The bunches were monitored daily for symptom expression of *B. cinerea*. Bunches of the sterile group, and

from the other unsterile group were used for isolation. From each bunch 10 rachis segments (25 mm), 20 laterals (20 mm), 40 pedicels and 120 berry skin segments (5 x 7 mm) (40 each from the pedicel-end, cheek and style-end) were removed. Five rachis, 10 laterals and 20 each of the pedicels and berry skin segments were placed in Petri dishes on Kerssies' *B. cinerea* selective medium (Kerssies, 1990), and five on a water agar medium supplemented with paraquat (Grindrat & Pezet, 1994). The plates were incubated at 22°C under diurnal light and the segments were monitored daily for symptom expression and the development of *B. cinerea*. After 11 days the number of segments yielding sporulating *B. cinerea* colonies were recorded, and the numbers used to quantify the amount of *B. cinerea* occurring at the various sites in the bunches.

In the 2000/2001 season, additional grape bunches were used to determine the efficacy of fungicides on pedicels and berries at different positions in the bunch at véraison and harvest stage. From each bunch, 10 berries with their pedicels positioned on the outer periphery of the bunch, and 10 berries with their pedicels positioned in the inner bunch near the rachis were removed. From these berries, 5 outer skin segments (the part of the berry which is exposed to the outer parts of the grape bunch) and 5 inner skin segments (berry part which is confined to the inner bunch parts), and their pedicels, were removed. The epidermal tissue segments (5 x 7 mm) and pedicels were placed on Petri dishes containing Kerssies' *B. cinerea* selective medium (Kerssies, 1990), or water agar medium supplemented with paraquat (Grindrat & Pezet, 1994). The plates were incubated at 22°C under diurnal light and the amount of *B. cinerea* occurring on the segments calculated as described previously.

The different treatments provided conditions that facilitated the development of *B. cinerea* by conidia on the surface of bunch tissue, or by latent mycelia in the tissue, during the period of incubation. Previous studies (Coertze & Holz, 1999; Coertze *et al*, 2001; Gütschow, 2001; Volkmann, 2001) with grape bunch tissue on Kerssies' medium showed that no superficial mycelial growth developed on the segments during the first 5 days of incubation. Hyphal growth usually occurred from cells underlying the cuticle into the medium after 5 days, which indicated direct penetration by conidia on the surface, or the development of latent mycelia from the host tissue during the incubation period. On segments from unsterile bunch parts, disease expression was therefore the result of infection by surface inoculum and the development of latent mycelia in host tissue. Decay incidences on segments from a specific site therefore gave an indication of infection at that site as

influenced by the amount of surface conidia and latent mycelia. Surface sterilization completely eliminated *B. cinerea* from the bunch surface (Sarig *et al.*, 1996; Coertze & Holz, 1999; Coertze *et al.*, 2001) and prevented infection by surface inoculum. Development of the pathogen from surface-sterilised bunch parts therefore gave an indication of infection at a specific site as influenced by latent mycelia. Paraquat terminated host resistance in the cells of the cuticular membrane without damaging host tissue, and thus facilitate the development of latent mycelia (Baur *et al.*, 1969; Cerkauskas & Sinclair, 1980; Pscheidt & Pearson, 1989; Grindrat & Pezet, 1994).

Statistical Analyses. The experimental design was a split plot repeated in 4 blocks. At full bloom stage, the main plot treatment was fungicides and the split treatment medium. The main plot treatments for all the other stages were fungicides and medium. The split treatment was sterilised and unsterile. Statistical computations were performed using SAS (Statistical Analyses System, 1990). The experiments were subjected to analyses of normality (Shapiro & Wilk, 1965). The data was examined further by using the analyses of variance (ANOVA) and the treatment means were compared using the Student's *t* LSD ($P = 0.05$) (Snedecor & Cochran, 1980).

RESULTS

Infection and fungicide efficacy on parts of grape bunches kept intact. At full bloom, clusters on unsprayed shoots of both cultivars were in both seasons highly susceptible to *B. cinerea* and developed symptoms of grey mould, which first appeared as small brown lesions on laterals and rachises and later on pedicels. The lesions eventually turned black and yielded sporulating colonies of *B. cinerea*. Sporulating colonies of the pathogen also developed on withered calyptras and stamens. Ovaries, however, remained green, with no symptoms of *B. cinerea* decay (Figs. 1-2). Clusters on shoots sprayed with fungicides remained asymptomatic during the 2 wk observation period. Bunches of both cultivars inoculated at the other growth stages remained asymptomatic in both seasons during the 2 wk observation period, irrespective of the treatment received.

Infection and fungicide efficacy on clusters used for isolation at full bloom. Table 1 shows the analysis of variance (ANOVA) for the effect of cultivar, fungicide treatment and medium on the percentage laterals on rachis sections that developed *B. cinerea* decay at full

bloom. Fungicide treatment had in both seasons and for both cultivars a highly significant effect ($P < 0.001$) on the percentage laterals yielding *B. cinerea*. The pathogen consistently developed from a high percentage laterals on rachis sections obtained from unsprayed bunches of both cultivars (Table 2). The fungicides were nearly equally effective in reducing the amount of *B. cinerea* on Dauphine clusters in 1999, and on Merlot in 2000. However, in 1999 on Merlot, the percentage infected laterals were significantly lower on clusters sprayed with pyrimethanil and cyprodinil/fludioxonil than with fenhexamid or iprodione. Furthermore, although the fungicides significantly reduced infection, nearly 16-37% of the laterals yielded the pathogen on the two media.

Infection and fungicide efficacy on parts of bunches used for isolation at pea size, bunch closure, véraison and at harvest. *Dauphine 1999/2000.* Surface-sterilisation almost completely eliminated *B. cinerea* at the various sites in both unsprayed and sprayed bunches. Furthermore, segments removed from the style-end of berries were for both sterility regimes virtually free from *B. cinerea.* Data from the sterile treatment, and those from the style-end of berries, were therefore not included in the statistical analysis. Table 3 shows the ANOVA for the effect of fungicide treatment, medium and infection site on *B. cinerea* incidence in unsterile bunches at pea size stage. Fungicide treatment, medium, infection site, and their interaction ($P < 0.01$) had a highly significant effect on *B. cinerea* incidence. Table 4 shows the ANOVA for the effect of fungicide treatment, medium and infection site on *B. cinerea* incidence in unsterile bunches at bunch closure, véraison and harvest stages. Fungicide treatment, infection site, and their interaction ($P < 0.01$) had a highly significant effect on *B. cinerea* incidence. For uniformity, the effects for the fungicide treatment x infection site interaction ($P = 0.001$) for the four growth stages are given in Table 5 using the mean values for the two media. The significant fungicide treatment x infection site interaction was due mainly to results obtained on the rachises and berry cheeks. At pea size stage in the unsprayed bunches, infection in rachises and laterals was at a significantly higher level than in the pedicels, the pedicel-end of the berry and the berry cheek. Rachises displayed the highest level of infection, while pedicels and the berry cheek had the least infection. Infection followed an almost similar pattern in the unsprayed bunches at bunch closure and véraison than at pea size stage. However, in the case of laterals, infection levels gradually declined from pea size to véraison. The infection pattern in the different sites of unsprayed bunches changed drastically at harvest. The pedicel-end of the berry had a significantly higher infection level than the other sites. Infection in the pedicel and berry cheek at harvest

was also at a higher level than at either pea size, bunch closure and véraison. Rachises and laterals, on the other hand, displayed a low infection level. The fungicides had a differential effect on infection at the various sites. In the case of rachises, infection was at each growth stage significantly reduced by each fungicide. In laterals, infection was significantly reduced at pea size and bunch closure. However, at these two sites, significant differences in efficacy were found between the fungicides at stages when infection levels were high. In such cases, infection was mostly not prevented. The fungicides were equally effective at sites that had low infection levels, and mostly prevented infection at these sites. In the pedicel-end of the berry, infection was significantly reduced at pea size and harvest. In the pedicels and berry cheeks, on the other hand, the fungicide spays caused no significant reduction in infection at pea size, bunch closure and véraison stages. Infection in these parts were only significantly reduced by fungicide application at harvest, when infection levels were higher at these sites in unsprayed bunches.

Dauphine 2000/2001. Segments removed from the style-end of berries were for both sterility regimes virtually free from *B. cinerea.* The pathogen however developed consistently from the other sites in bunches of both sterility regimes. Data of the the style-end of berries were therefore not included in the statistical analysis. Table 6 shows the ANOVA for the effect of fungicide treatment, sterility regime, medium and infection site on *B. cinerea* incidence in bunches at pea size to harvest stage. Fungicide treatment, infection site ($P < 0.01$), and the interaction fungicide treatment x sterility regime x infection site ($P < 0.01$) had a highly significant effect on *B. cinerea* incidence. Several general responses can be derived from the significant effect of the interaction. In the unsprayed, unsterile bunches infection levels were high in rachises at pea size stage, bunch closure and véraison (Table 7). Infection was relatively high in the laterals of unsprayed, unsterile bunches at pea size stage, and high at this site at véraison and harvest. In the pedicels and berry cheek, on the other hand, infection was constantly low at all the growth stages. Infection levels however increased dramatically at the pedicel end of the berry of unsprayed, unsterile bunches at harvest. Surface sterilisation mostly caused a significant reduction in infection at sites that had high infection in the unsprayed bunches. The different fungicides effectively reduced *B. cinerea* infection in the rachises, laterals and the pedicel end of the berry on unsterile bunches. In the case of rachises, infection was significantly reduced by each fungicide at pea size, bunch closure and véraison. In laterals, infection was significantly reduced at pea size, véraison and at harvest. In the pedicel-end of the berry, infection was significantly reduced at

véraison and at harvest. In the bunches that were surface sterilised, no meaningful differences were found in infection at the various sites between bunches of the different treatments.

Merlot 1999/2000. Surface-sterilisation nearly completely eliminated *B. cinerea* at the various sites in both unsprayed and sprayed bunches. Furthermore, segments removed from the style-end of berries were for both sterility regimes virtually free from *B. cinerea.* Data of the sterile treatment, and those of the style-end of berries, were therefore not included in the statistical analysis. Table 8 shows the ANOVA for the effect of fungicide treatment, medium and infection site on *B. cinerea* incidence in unsterile bunches at pea size stage. Fungicide treatment, infection site ($P < 0.01$), and their interaction ($P = 0.0042$) had a highly significant effect on *B. cinerea* incidence. Table 9 shows the ANOVA for the effect of fungicide treatment, medium and infection site on *B. cinerea* incidence in unsterile bunches at bunch closure, véraison and harvest stages. Fungicide treatment, infection site ($P < 0.01$), and their interaction ($P = 0.0005$) had a highly significant effect on *B. cinerea* incidence. In the unsprayed bunches infection levels were constantly high in the rachises and laterals at pea size stage, bunch closure and at harvest (Table 10). On the other hand, infection was constantly low during pea size and bunch closure in the pedicels, the pedicel end of the berry and the berry cheek, but levels in these parts were high at harvest. The different fungicides reduced *B. cinerea* infection in the rachises and laterals at pea size, bunch closure and harvest stages. However, infection at these two sites was mostly not prevented. The fungicide also caused a significant reduction in infection in the pedicels, the pedicel-end of the berry and the berry cheek when applied at harvest. However, the fungicides had no effect on infection in these parts when applied to the bunches at pea size and bunch closure stages.

Merlot 2000/2001. Segments removed from the style-end of berries were for both sterility regimes virtually free from *B. cinerea.* The pathogen however developed consistently from the other sites in bunches of both sterility regimes. Data of the the style-end of berries were therefore not included in the statistical analysis. Table 11 shows the ANOVA for the effect of fungicide treatment, sterility regime, medium and infection site on *B. cinerea* incidence in bunches at pea size to harvest stage. Fungicide treatment, infection site ($P < 0.01$), and the interaction fungicide treatment x infection site ($P < 0.01$) had a highly significant effect on *B. cinerea* incidence at all the gowth stages. In addition, sterility regime had a significant effect ($P < 0.01$) on *B. cinerea* incidence at pea size, bunch closure

and the harvest stage. Infection at the various sites, and fungicide efficacy, followed a nearly similar pattern to that found on Dauphine and Merlot during the previous seasons. Infection levels recorded at the various sites in bunches are given in Table 12.

Infection and fungicide efficacy on pedicels and berries at different positions in the bunch. The ANOVA for the effect of fungicide treatment, sterility regime, medium, infection site and position shows that the fungicide treatment x sterility regime x infection site interaction had a highly significant effect ($P < 0.01$) on *B. cinerea* incidence on Dauphine at véraison and harvest (Table 13). On Merlot, the ANOVA for the effect of fungicide treatment, sterility regime, medium, site and position shows the fungicide treatment x site interaction had a highly significant effect ($P < 0.01$) on *B. cinerea* incidence at the two growth stages (Table 14). However, in both cultivars no significant differences were found in infection levels between pedicels, or berry cheeks, obtained from different positions in fungicide treated bunches.

DISCUSSION

The evaluation of fungicide efficacy in commercial vineyards can be influenced by the sporadic occurrence of *B. cinerea* in grape bunches (De Kock & Holz, 1991; Holz *et al.*, 1997, 1998; Volkmann, 2001), differences in structure during bunch development (Thwaite, 2001), and the phenomenon that symptom expression in grape bunches is governed by the resistance reaction of the various bunch parts (Coertze & Holz, 1999; Gütschow, 2001). Grape bunches from various phenological stages ,were therefore sprayed with fungicides in a spray chamber or left untreated, and inoculated with dry, airborne *B. cinerea* conidia in a spore settling tower. Infection and fungicide efficacy was determined by observing intact bunches for symptom expression, and by estimating the amount of *B. cinerea* at various sites in bunches with isolation studies. The study showed that dry, airborne *B. cinerea* conidia, and the fungicide sprays, penetrated loose and tight clustered bunches from bloom to harvest and evenly landed on the various bunch parts. At full bloom, the amount of *B. cinerea* in unsprayed bunches was high on the laterals and pedicels, but low on the embryos. Unsprayed intact bunches at full bloom were highly susceptible to *B. cinerea* and developed symptoms of grey mould. The fungicides inhibited symptom expression at full bloom, but could not prevent infection. Unsprayed bunches inoculated at the pea size, bunch closure, véraison and harvest stages, on the other hand, remained asymptomatic. The amount of *B. cinerea* was

generally high in the rachises and laterals at pea size and bunch closure stages, and in the pedicel-end of the berries at harvest. Infection was constantly low in the berry cheek, whereas the style-end of the berry was virtually free of the pathogen. The fungicides had a differential effect on infection at the various sites. In the case of rachises, the amount of *B. cinerea* was at each growth stage drastically reduced by each fungicide. In laterals, it was effectively reduced at pea size and bunch closure. However, at these two sites, significant differences in efficacy were found between the fungicides at stages when the amount of *B. cinerea* was high. In such cases infection was mostly not prevented. The fungicides were equally effective at sites that had low amounts of *B. cinerea*. In spite of these differences, the fungicides, which belong to different chemical classes, at all growth stages completely prevented symptom expression in the intact bunches.

Laboratory studies showed (Holz *et al.*, 1997, 1998; Coertze *et al.*, 2001; Holz & Calitz, 2001; Gütschow, 2001) that although grape bunches from commercial vineyards in South Africa do not normally develop grey mould during pea size and bunch closure, they may carry high amounts of *B. cinerea* in their structural parts, and not on their berries. These findings imply that incipient infections can cause both mid- or late-season bunch rot following a period of fungal latency in the rachis, laterals or pedicels, and not in berry cheeks and style-ends. By using a differential set of skin segment isolation, paraquat treatment, and freezing techniques on sterile and non-sterile bunches, it was shown (Du Preez, 2001; Holz & Calitz, 2001; Volkmann, 2001) that the pathogen at these growth stages mostly occurred superficially at the different sites in bunches, and seldom as latent mycelia in the bunch tissue. The fact that surface-sterilisation almost completely eliminated *B. cinerea* at the corresponding growth stages at the various sites in bunches that were exposed to dry, airborne conidia, substantiated these findings on the characteristic pattern of natural occurrence of *B. cinerea* in grape bunches. The different fungicides caused a similar reduction in the amount of *B. cinerea*, which proved their high protective ability and efficacy to reduce superficial *B. cinerea* inoculum at the various sites in immature and mature grape bunches. The finding that the fungicides all meaningfully reduced the amount of *B. cinerea* in bunches at full bloom and prevented symptom expression, is invaluable in planning strategies for disease control. Prevention of symptom development is an essential step in the reduction of secondary inoculum in the vineyard, and the control of *B. cinerea* epiphytotics.

Little is known about the relation between conidial density, infection and subsequent Botrytis berry rot on grape. In most studies where grape berries were artificially inoculated, suspensions containing $\geq 10^4$ conidia per ml were used as a standard procedure to induce symptom expression (Nelson, 1951; Nair, 1985; Marois *et al.*, 1986; Nair *et al.*, 1988; Thomas *et al.*, 1988; Avissar & Pessis, 1991; De Kock & Holz, 1991; Broome *et al.*, 1995; Chardonnet *et al.*, 1997). Warren *et al.* (1999) showed that suspensions containing $\geq 10^4$ conidia per ml were required on on-vine inflorescences and bunches of the highly susceptible wine grape cultivar Chardonnay to induce symptom expression on frozen inflorescences or berries. Hill *et al.* (1981) calculated that from a total of 3500 conidia per 15.9 mm^2 cuticle surface only 1-2 conidia were able to penetrate the isolated cuticle layer. Coertze and Holz (1999) studied the infectivity of single airborne *B. cinerea* conidia, and showed that symptom expression is not governed by conidium density on berries. Coertze *et al.* (2001) subsequently proved that infections in grape berry cheeks established by this infection mode were few. The findings of these workers suggest that disease expression by *B. cinerea* on grape berries is not dependent upon inoculum density. This study showed that this hypothesis may also hold for the expression of *B. cinerea* symptoms on the structural parts of grape bunches. Since symptom expression often occur in vineyards (Nair, 1985; Nair & Hill, 1992), these findings suggest that the role of predisposing factors, such as wounding may be underestimated in the epidemiology of *B. cinerea*. Grape berries can be wounded by insects, frost, hail, windblown sand, sun, or rapid water intake leading to splitting (Jarvis, 1980; Savage & Sall, 1983).

Most producers appeared to be adhering to recommendations (Pearson & Riegel, 1983; Wang & Coley-Smith, 1986; Nair *et al.*, 1987; Northover, 1987; De Kock & Holz, 1991,1994; LeRoux, 1995) that advocate four applications per season, namely at the end of flowering, at bunch closure, at véraison and three weeks before harvest. However, recent findings on the natural occurrence of *B. cinerea* in South African vineyards, and data on the infectivity of dry, airborne conidia, indicate that the advocated schedule of fungicide application should be reconsidered. According to these findings, blades of young grape leaves normally carry high levels of latent, natural *B. cinerea* infection (Gütschow, 2001), which can act as an important source for primary infection of young infloresences before bloom. Infloresences on vinelets and vineyard shoots proved to be susceptible to *B. cinerea* shortly after budburst (Gütschow, 2001; Part 2). Furthermore, the amount of *B. cinerea* in bunches is generally the highest shortly after bloom, and lowest prior to harvest (Gütschow,

2001). This study showed that bunches exposed to airborne conidia developed symptoms of grey mould at full bloom, but remained asymptomatic at the pea size, bunch closure, véraison and harvest stages. Thus, to lower the amount of inoculum on the surface of inner bunch parts, prevent infection, symptom expression and the build-up of *B. cinerea* inoculum in grapevine, three preventative applications are recommended to reduce primary infection events: (a) between budding and prebloom to counteract primary infection of young leaves and inflorescences (b) during flowering to prevent pimary infection of the highly susceptible rachises, laterals and pedicels; (c) at bunch closure, to prevent infection of the inner bunch parts, especially for cultivars with tight bunches. The fungicides used in this study should effectively reduce the amount of *B. cinerea* on leaves, petioles, nodes, internodes and inflorescenses, and prevent infection (Part 2). This study showed that if these fungicides are applied properly to the bunches, they should effectively penetrate and cover the inner bunch parts, reduce the amount of *B. cinerea* at the various sites in the bunches, and prevent infection and symptom expression at all growth stages. Therefore, more work is needed to evaluate fungicide application techniques by conventional spraying methods for proper coverage of bunches.

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Table 1. Analysis of variance of data for the effect of fungicide treatment and medium on the percentage laterals on grape rachis sections that developed *Botrytis cinerea* at full bloom in two seasons

		Dauphine		Merlot	
Source of Variation	**DF**	**MS**	**SL**	**MS**	**SL**
Season one (1999/2000)					
Replicate	4	515.80		2000.75	
Fungicide Treatment (FT)	4	31,335.35	0.0001	32629.04	0.0001
Error(FT)	16	685.96		862.74	
Medium (Med)	1	221.76	0.6707	1315.49	0.2556
FT*Med	4	469.34	0.8104	1457.36	0.2350
Error(FT x Med)	20	1,190.70		960.39	
Error(FT x Med x Part)	200	1,142.11		1551.23	
Season two (2000/2001)					
Replicate	3	3252.70		825.38	
Fungicide Treatment (FT)	5	50137.50	0.0001	59203.62	0.0001
Error(FT)	15	1158.26		3267.51	
Medium (Med)	1	6020.83	0.0982	1045.28	0.4267
FT x Med	5	1070.83	0.7429	520.28	0.8887
Error(FT x Med)	18	1979.55		1580.39	
Error(FT x Med x Part)	432	1120.08		1263.17	

Table 2. Mean[w] percentage laterals on rachis sections, isolated from grape clusters sprayed with fungicides and inoculated with dry, airborne conidia of *Botrytis cinerea* at full bloom that yielded the pathogen on two different media[x]

Fungicide	**Dauphine**		**Merlot**	
Treatment	**Year 1[y]**	**Year 2[z]**	**Year 1**	**Year 2**
Cyprodinil/Fludioxonil	28.0 b	14.0 b	22.0 c	24.0 b
Iprodione	30.0 b	18.0 b	37.0 b	25.0 b
Fenhexamid	29.0 b	20.0 b	37.0 b	29.0 b
Pyrimethanil	27.0 b	16.3 b	23.0 c	21.3 b
Control	84.2 a	76.3 a	84.3 a	89.0 a
Uninoculated Control		10.0 b		16.3 b

[w]Values in each column followed by the same letter are not significantly different according to the Student's *t*-test at P = 0.05.

[x]Rachis sections were incubated on Kerssies and paraquat media. Values averaged over media.

[y]Year 1 = season 1999/2000.

[z]Year 2 = season 2000/2001.

Table 3. Analysis of variance of data for the effect of fungicide treatment, medium and infection site on the percentage bunch parts isolated from unsterile Dauphine grape bunches that yielded *Botrytis cinerea* at pea size in the 1999/2000 season

Source of Variation	Df	SS	MS	F Value	SL
Replicate	2	0.400	0.200	0.010	0.9886
Fungicide Treatment (FT)	4	2,092.356	523.089	30.020	0.0001
Error(FT)	8	139.378	17.422		
Medium (Med)	1	49.089	49.089	8.460	0.0156
FT x Med	4	811.911	202.977	35.000	0.0001
Error(FT x Med)	10	58.000	5.800		
Infection Site (IS)	5	2,854.333	570.867	21.480	0.0001
FT x IS	20	3,180.444	159.022	5.980	0.0001
Med x IS	5	181.444	36.289	1.370	0.2439
FT x Med x IS	20	2,018.222	100.911	3.800	0.0001
Error(FT x Med x IS)	100	2,658.222	26.582		
Corrected Total	239		78.456		

Table 4. Analysis of variance of data for the effect of fungicide treatment, medium and infection site on the percentage bunch parts isolated from unsterile Dauphine grape bunches that yielded *Botrytis cinerea* at bunch closure, véraison and harvest in the 1999/2000 season

		Bunch Closure		Véraison		Harvest	
Source of Variation	Df	MS	SL	MS	SL	MS	SL
Replicate	3	58.800	0.4328	27.972	0.2061	17.467	0.3018
Fungicide Treatment (FT)	4	573.567	0.0010	162.791	0.0007	240.108	0.0001
Error(FT)	12	59.744		15.792		12.842	
Medium (Med)	1	21.600	0.3973	40.016	0.3929	52.267	0.1954
FT x Med	4	14.433	0.7311	45.475	0.4995	6.558	0.9170
Error(FT x Med)	15	28.444		51.717		28.456	
Infection Site (IS)	5	514.400	0.0001	279.936	0.0001	93.067	0.0001
FT x IS	20	163.567	0.0001	69.511	0.0007	38.808	0.0001
Med x IS	5	34.400	0.6832	10.816	0.8510	30.667	0.0103
FT x Med x IS	20	11.633	0.9999	32.475	0.2714	19.858	0.0090
Error(FT x Med x IS)	150	55.307		27.319		9.804	
Corrected Total	239	76.308		39.806		20.648	

Table 5. Means of the effect of interaction fungicide treatment x infection site on the percentage bunch parts isolated from unsterile Dauphine grape bunches that yielded *Botrytis cinerea* on the two media[x] in the 1999/2000 season

	Bunch parts[y] infected by *B. cinerea* (%)																			
Fungicide	**Pea Size**					**Bunch Closure**					**Véraison**					**Harvest**				
Treatment	**R**	**L**	**P**	**PE**	**C**	**R**	**L**	**P**	**PE**	**C**	**R**	**L**	**P**	**PE**	**C**	**R**	**L**	**P**	**PE**	**C**
Inoculated Control	30.0 a	18.3 b	3.3 cde	8.3 c	2.0 de	30.0 a	12.5 b	3.5 cd	4.5 cd	3.5 cd	20.0 a	5.0 b	0.5 b	2.5 b	2.3 b	5.0 bc	0.0 d	8.0 b	13.5 a	7.5 b
Iprodione	3.3 cde	8.3 c	0.6 e	1.0 de	0.0 e	5.0 cd	0.0 d	1.0 d	1.0 d	0.5 d	5.0 b	0.0 b	0.5 b	1.0 b	0.5 b	0.0 d	2.5 cd	1.0 d	3.0 cd	1.8 d
Cyprodinil/ fludioxonil	6.6 cd	0.0 e	0.3 e	1.0 de	0.0 e	0.0 d	0.0 d	1.5 d	1.5 d	0.5 d	5.0 b	2.5 b	0.0 b	0.0 b	0.3 b	0.0 d	0.0 d	0.5 d	1.0 d	0.0 d
Pyrimethanil	0.0 e	6.6 cd	0.0 e	1.3 de	0.0 e	5.0 cd	0.0 d	2.0 d	2.0 d	0.0 d	0.0 b	2.5 b	0.0 b	0.0 b	0.5 b	0.0 d	0.0 d	0.0 d	1.0 d	2.5 cd
Fenhexamid	3.3 cde	3.3cde	0.0 e	0.3 e	0.6 e	10.0 bc	0.0 d	0.5 d	1.5 d	1.0 d	5.0 b	0.0 b	0.0 b	0.0 b	0.8 b	0.0 d	0.0 d	0.5 d	1.5 d	2.8 cd

[x]Parts, obtained from bunches sprayed with fungicides and inoculated with dry, airborne *Botrytis cinerea* conidia, were incubated on Kerssies and paraquat medium. Values averaged over media.

[y]R = rachis, L = laterals, P = pedicel, PE = pedicel end of berry, C = berry cheek.

[z]Values in each column followed by the same letter are not significantly different according to the Student's *t*-test at P = 0.05.

Table 6. Analysis of variance of data for the effect of fungicide treatment, sterility regime, medium and infection site on the percentage bunch parts isolated from Dauphine grape bunches that yielded *Botrytis cinerea* at different phenological stages in the 2000/2001 season

		Pea Size		Bunch Closure		Véraison		Harvest	
Source of Variation	**Df**	**MS**	**SL**	**MS**	**SL**	**MS**	**SL**	**MS**	**SL**
Replicate	3	24.421	0.2077	10.474	0.6368	59.186	0.3965	14.279	0.4084
Fungicide Treatment (FT)	5	385.277	0.0001	442.881	0.0001	667.126	0.0001	372.994	0.0001
Error(FT)	15	14.282		18.044		55.992		13.898	
Sterility Regime (SR)	1	487.673	0.0005	8.506	0.6819	937.890	0.0021	1,097.266	0.0001
FT x SR	5	181.631	0.0011	10.173	0.9550	404.349	0.0028	228.619	0.0001
Error(FT x SR)	18	27.083		49.016		72.439		9.042	
Medium (Med)	1	91.840	0.0144	14.062	0.4742	244.140	0.0010	257.335	0.0050
FT x Med	5	27.880	0.1010	4.479	0.9732	177.682	0.0001	46.605	0.1802
SR x Med	1	84.027	0.0188	39.062	0.2359	257.335	0.0008	231.293	0.0075
FT x SR x Med	5	8.611	0.6854	46.354	0.1540	136.085	0.0001	37.855	0.2799
Error(FT x SR x Med)	36	13.889		26.881		19.170		28.805	
Infection Site (IS)	5	133.506	0.0001	68.923	0.0001	126.710	0.0015	225.286	0.0001
FT x IS	25	79.423	0.0001	56.673	0.0001	92.001	0.0001	100.390	0.0001
SR x IS	5	74.652	0.0001	38.923	0.0122	74.557	0.0397	142.786	0.0001
FT x SR x IS	25	62.361	0.0001	29.215	0.0008	72.140	0.0005	52.265	0.0001
Med x IS	5	21.527	0.1095	9.479	0.6069	37.890	0.3080	32.439	0.1250
FT x Med x IS	25	12.819	0.3636	8.270	0.9174	59.057	0.0077	15.585	0.6960
SR x Med x IS	5	35.381	0.0119	15.729	0.3091	47.335	0.1894	32.230	0.1275
FT x SR x Med x IS	25	4.715	0.9964	17.645	0.1271	45.210	0.0848	16.668	0.6154
Error(FT x SR x Med x IS)	360	11.875		13.119		31.583		18.660	
Corrected Total	575	25.285		22.463		53.720		34.733	

Table 7. Means of the effect of interaction fungicide treatment x sterility regime x infection site on the percentage bunch parts isolated from Dauphine grape bunches that yielded *Botrytis cinerea* on the two media[x] in the 2000/2001 season

	Bunch Parts[y] infected by *B. cinerea* (%)																					
	Pea Size					Bunch closure					Véraison					Harvest				
Treatment	R	L	P	PE	C	R	L	P	PE	C	R	L	P	PE	C	R	L	P	PE	C
Inoculated Control																				
NS	27.5 a	15.0 b	2.5 cde	5.0 c	5.0 c	17.5 a	6.2 c	3.8 de	6.3 c	3.1 def	17.5 b	28.8 a	5.0 cde	16.3 b	5.6 cd	2.5 e-h	28.8 a	4.4 d-g	15.0 b	6.9 cd
S	2.5 cde	3.7 cd	0.6 de	2.5 cde	1.3 de	5.0 cd	13.7 b	1.9 def	6.3 c	3.8 de	5.0 cde	1.3 de	0.6 de	1.9 de	0.6 de	0.0 h	5.0 def	0.0 h	1.3 fgh	0.6 gh
Iprodione																				
NS	2.5 cde	2.5 cde	0.6 de	1.3 de	0.6 de	0.0 f	0.0 f	0.0 f	0.0 f	0.0 f	7.5 c	0.0 e	3.1 cde	5.6 cd	2.5 cde	2.5 e-h	6.3 cde	1.3 fgh	2.5 e-h	1.9 fgh
S	0.0 e	0.0 e	0.0 e	0.0 e	0.6 de	0.0 f	0.0 f	0.0 f	0.0 f	0.0 f	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 h	0.0 h	0.0 h	0.6 gh	0.0 h
Cyprodinil/ fludioxonil																				
NS	2.5 cde	0.0 e	0.6 de	2.5 cde	1.3 de	0.0 f	0.0 f	0.0 f	1.3 ef	0.6 ef	0.0 e	0.0 e	0.0 e	1.3 de	0.0 e	0.0 h	0.0 h	1.3 fgh	1.3 fgh	0.0 h
S	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 f	0.0 f	0.0 f	0.0 f	0.0 f	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 h	0.0 h	0.0 h	0.0 h	0.0 h
Pyrimethanil																				
NS	0.0 e	0.0 e	0.0 e	2.5 cde	0.0 e	0.0 f	0.0 f	0.6 ef	1.3 ef	2.5 def	0.0 e	0.0 e	0.6 de	1.3 de	1.3 de	0.0 h	0.0 h	1.3 fgh	1.9 fgh	1.3 fgh
S	0.0 e	1.3 de	0.0 e	0.0 e	0.0 e	0.0 f	0.0 f	0.0 f	1.3 ef	1.3 ef	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 h	0.0 h	0.0 h	0.0 h	0.0 h
Fenhexamid																				
NS	0.0 e	1.3 de	1.3 de	1.3 de	1.3 de	0.0 f	0.0 f	0.6 ef	1.3 ef	1.3 ef	0.0 e	0.0 e	0.6 de	1.3 de	0.6 de	0.0 h	0.0 h	0.6 gh	2.5 e-h	1.3 fgh
S	2.5 cde	0.0 e	0.6 de	0.0 e	0.0 e	0.0 f	0.0 f	0.0 f	0.0 f	0.0 f	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 h	0.0 h	0.0 h	0.0 h	0.0 h
Uninoculated Control																				
NS	2.5 cde	1.3 de	1.3 de	0.6 de	0.6 de	0.0 f	1.3 ef	1.3 ef	3.1 def	0.6 ef	0.0 e	1.3 de	1.3 de	2.5 cde	0.0 e	5.0 def	10 c	1.9 fgh	6.9 cd	0.0 h
S	0.0 e	0.0 e	0.6 de	0.6 de	0.0 e	0.0 f	5.0 cd	0.6 ef	3.1 def	3.1 def	0.0 e	2.5 cde	0.0 e	0.0 e	0.0 e	0.0 h	0.0 h	0.0 h	0.0 h	0.0 h

[x]Parts, obtained from bunches sprayed with fungicides and inoculated with dry, airborne *Botrytis cinerea* conidia, were incubated on Kerssies and paraquat medium. Values averaged over media.
[y]R = rachis, L = laterals, P = pedicel, PE = pedicel end of berry, C = berry cheek.
[z]Values in each column followed by the same letter are not significantly different according to the Student's *t*-test at P = 0.05.

Table 8. Analysis of variance of data for the effect of fungicide treatment, medium and infection site on the percentage bunch parts isolated from unsterile Merlot grape bunches that yielded *Botrytis cinerea* at pea size in the 1999/2000 season

Source of Variation	Df	SS	MS	F Value	SL
Replicate	2	64.711	32.355	0.430	0.6642
Fungicide Treatment (FT)	4	5029.689	1257.422	16.74	0.0006
Error(FT)	8	600.844	75.106		
Medium (Med)	1	18.689	18.689	0.160	0.7015
FT x Med	4	23.644	5.911	0.050	0.9947
Error(FT x Med)	10	1200.667	120.067		
Infection Site (IS)	5	4431.578	886.315	8.490	0.0001
FT x IS	20	4735.644	236.782	2.270	0.0042
Med x IS	5	15.044	3.008	0.030	0.9996
FT x Med x IS	20	271.289	13.564	0.130	1.0000
Error(FT x Med x IS)	100	10443.111	104.431		
Corrected Total	239		149.915		

Table 9. Analysis of variance of data for the effect of fungicide treatment, medium and infection site on the percentage bunch parts isolated from unsterile Merlot grape bunches that yielded *Botrytis cinerea* at different phenological stages in the 1999/2000 season

		Bunch Closure		Véraison		Harvest	
Source of Variation	Df	MS	SL	MS	SL	MS	SL
Replicate	3	139.688	0.3865	27.838	0.4991	97.44	0.5414
Fungicide Treatment (FT)	4	1287.233	0.0009	300.067	0.0013	4157.142	0.0001
Error(FT)	12	126.856		33.256		129.375	
Medium (Med)	1	1.067	0.8943	120.416	0.2211	516.267	0.0537
FT x Med	4	24.567	0.7913	24.167	0.8554	172.141	0.2629
Error(FT x Med)	15	58.4		73.861		117.767	
Infection Site (IS)	5	468.586	0.0001	197.056	0.0001	847.746	0.0001
FT x IS	20	341.353	0.0001	50.906	0.0062	238.521	0.0005
Med x IS	5	52.426	0.3584	26.576	0.3636	113.226	0.2949
FT x Med x IS	20	59.726	0.2128	17.326	0.805	60.401	0.8601
Error(FT x Med x IS)	150	47.298		24.190		91.578	
Corrected Total	239	107.895		38.155		192.321	

Table 10. Means of the effect of the interaction fungicide treatment x infection site on the percentage bunch parts isolated from unsterile Merlot grape bunches that yielded *Botrytis cinerea* on the two media[x] in the 1999/2000 season

	Bunch parts[y] infected by *B. cinerea* (%)																			
	Pea Size					Bunch Closure					Véraison					Harvest				
Treatment	R	L	P	PE	C	R	L	P	PE	C	R	L	P	PE	C	R	L	P	PE	C
Inoculated Control	36.7 a	35.0 a	7.7 bc	9.7 bc	4.7 bc	40.0 a	20.0 b	6.5 c	6.5 c	5.0 c	15.0 a	7.5 b	6.5 bc	13.0 a	2.8 b-e	30.0 a	32.5 a	19.0 b	33.0 a	35.5 a
Iprodione	6.7 bc	5.0 bc	1.3 c	2.3 bc	1.0 c	5.0 c	0.0 c	3.0 c	2.5 c	1.0 c	0.0 e	2.5 cde	2.5 cde	5.0 bcd	0.3 de	5.0 cd	10.0 bc	3.5 cd	6.5 cd	6.3 cd
Cyprodinil/ fludioxonil	13.3 b	1.7 bc	1.3 c	1.3 c	0.0 c	0.0 c	0.0 c	2.0 c	2.0 c	1.0 c	5.0 bcd	5.0 bcd	2.5 cde	3.5 b-e	0.3 de	10.0 bc	2.5 cd	2.0 cd	4.5 cd	4.5 cd
Pyrimethanil	10.0 bc	1.7 bc	1.3 c	2.0 bc	0.0 c	5.0 c	0.0 c	3.0 c	2.0 c	0.5 c	0.0 e	0.0 e	3.5 b-e	5.0 bcd	0.8 de	5.0 cd	5.0 cd	3.5 cd	5.0 cd	4.5 cd
Fenhexamid	3.3 bc	3.3 bc	1.3 c	1.3 c	0.0 c	0.0 c	2.5 c	2.0 c	2.0 c	1.0 c	0.0 e	2.5 cde	3.0 b-e	4.5 b-e	1.3 de	0.0 d	10.0 bc	2.5 cd	6.0 cd	5.3 cd

[x]Parts, obtained from bunches sprayed with fungicides and inoculated with dry, airborne *Botrytis cinerea* conidia, were incubated on Kerssies and paraquat medium. Values averaged over media.
[y]R = rachis, L = laterals, P = pedicels, PE = pedicel end of berry, C = berry cheek.
[z]Values in each column followed by the same letter are not significantly different according to the Student's *t*-test at P = 0.05.

Table 11. Analysis of variance of data for the effect of fungicide treatment, sterility regime, medium and infection site on the percentage bunch parts isolated from Merlot grape bunches that yielded *Botrytis cinerea* at different phenological stages in the 2000/2001 season

		Pea Size		Bunch Closure		Véraison		Harvest	
Source of Variation	**Df**	**MS**	**SL**	**MS**	**SL**	**MS**	**SL**	**MS**	**SL**
Replicate	3	22.381	0.5199	106.770	0.3420	9.649	0.2862	58.376	0.3090
Fungicide Treatment (FT)	5	501.328	0.0001	1,341.215	0.0001	390.980	0.0001	967.855	0.0001
Error(FT)	15	28.458		88.646		6.976		44.731	
Sterility Regime (SR)	1	206.640	0.0027	1,914.063	0.0001	0.391	0.9025	1,016.015	0.0001
FT x SR	5	67.994	0.0134	635.104	0.0001	14.036	0.7330	362.369	0.0001
Error(FT x SR)	18	17.144		66.898		25.304		35.894	
Medium (Med)	1	53.168	0.0524	321.006	0.0001	3.515	0.6498	121.918	0.0042
FT x Med	5	39.730	0.2280	183.090	0.0001	0.911	0.9980	38.689	0.0241
SR x Med	1	36.501	0.1051	108.506	0.0050	9.765	0.4503	22.960	0.1928
FT x SR x Med	5	9.314	0.6233	64.340	0.0010	6.953	0.8354	35.564	0.0344
Error(FT x SR x Med)	36	13.209		12.153		16.768		13.035	
Infection Site (IS)	5	95.182	0.0001	401.319	0.0001	75.355	0.0002	197.543	0.0001
FT x IS	25	99.619	0.0001	158.028	0.0001	74.668	0.0001	135.105	0.0001
SR x IS	5	20.182	0.3677	200.625	0.0027	31.953	0.0613	98.515	0.0002
FT x SR x IS	25	20.786	0.3182	105.667	0.0045	16.223	0.3614	58.494	0.0001
Med x SR	5	21.293	0.3358	60.486	0.3486	6.744	0.8136	17.126	0.4988
FT x Med x SR	25	15.105	0.7260	29.569	0.9639	3.015	1.0000	8.022	0.9954
SR x Med x IS	5	5.043	0.9286	25.069	0.8025	8.411	0.7302	13.376	0.6371
FT x SR x Med x IS	25	3.355	1.0000	29.402	0.9651	1.723	1.0000	10.605	0.9667
Error(FT x SR x Med x IS)	360	18.585		53.947		15.003		19.604	
Corrected Total	575	27.021		82.919		20.304		41.983	

Table 12. Means of the effect of the interaction fungicide treatment x infection site on the percentage bunch parts isolated from Merlot grape bunches that yielded *Botrytis cinerea* on the two media[x] in the 2000/2001 season

	Bunch Parts[y] infected by *B. cinerea* (%)																			
	Pea Size					Bunch Closure					Véraison					Harvest				
Treatment	R[a]	L[b]	P[c]	PE[d]	C[e]	R	L	P	PE	C	R	L	P	PE	C	R	L	P	PE	C
Inoculated Control	16.25 a	9.37 b	2.8 cd	5.3 c	1.9 d	18.8 a	20.0 a	4.0 cd	11.6 b	6.3 c	8.8 b	13.1 a	1.3 c	6.6 b	1.6 c	10.0 b	20.0 a	3.8 cd	11.9 b	5.9 c
Iprodione	1.3 d	0.0 d	0.6 d	0.6 d	0.3 d	6.3 c	3.8 cd	1.3 cd	3.1 cd	1.9 cd	0.0 c	0.0 c	0.0 c	2.5 c	0.9 c	0.0 e	1.3 de	1.3 de	3.8 cd	2.8 de
Cyprodinil/ fludioxonil	0.0 d	0.0 d	0.6 d	0.3 d	0.3 d	0.0 cd	0.0 d	0.0 d	1.6 cd	0.6 d	0.0 c	0.0 c	0.3 c	0.9 c	0.6 c	0.0 e	0.0 e	0.6 e	1.6 de	0.3 e
Pyrimethanil	0.0 d	0.0 d	0.3 d	1.3 d	0.6 d	1.3 cd	0.0 d	0.6 d	1.3 cd	0.9 d	0.0 c	0.0 c	0.0 c	0.6 c	0.3 c	1.3 de	0.6 e	2.2 de	2.8 de	1.3 de
Fenhexamid	0.0 d	0.0 d	1.3 d	0.6 d	0.3 d	1.3 cd	0.6 d	0.0 d	0.9 d	0.6 d	0.0 c	0.0 c	0.6 c	0.3 c	0.6 c	0.0 e	0.0 e	0.3 e	1.6 de	0.9 de
Uninoculated Control	0.0 d	0.0 d	0.0 d	1.3 d	0.6 d	5.0 cd	1.9 cd	1.3 cd	1.6 cd	0.6 d	0.0 c	0.0 c	0.0 c	0.6 c	0.0 c	2.5 de	1.3 de	0.0 e	0.0 e	0.6 e

[x]Parts, obtained from bunches sprayed with fungicides and inoculated with dry, airborne *Botrytis cinerea* conidia, were incubated on Kerssies and paraquat medium. Values averaged over media.
[y]R= rachis, L = laterals, P = pedicels, PE = pedicel end of berry, C = berry cheek.
[z]Values in each column followed by the same letter are not significantly different according to the Student's *t*-test at P = 0.05.

Table 13. Analysis of variance of data for the effect of fungicide treatment, sterility regime, medium, infection site and position on the percentage berry parts isolated from Dauphine grape bunches that yielded *Botrytis cinerea* at different phenological stages in the 2000/2001 season

		Véraison		Harvest	
Source of Variation	**DF**	**MS**	**SL**	**MS**	**SL**
Replicate	3	9.549	0.4860	1.201	0.8753
Fungicide Treatment (FT)	5	179.861	0.0001	81.398	0.0001
Error(FT)	15	11.181		5.263	
Sterility Regime (SR)	1	802.778	0.0001	408.377	0.0001
FT x SR	5	121.215	0.0001	55.356	0.0001
Error(FT)	18	11.082		4.239	
Medium (Med)	1	56.250	0.0157	41.710	0.0016
FT x Med	5	16.563	0.1194	9.939	0.0329
SR x Med	1	29.340	0.0752	27.127	0.0094
FT x SR x Med	5	11.736	0.2687	6.606	0.1309
Error(FT x SR x Med)	36	8.738		3.602	
Infection Site (IS)	2	447.179	0.0001	257.856	0.0001
FT x IS	10	54.731	0.0001	35.148	0.0001
SR x IS	2	347.700	0.0001	213.845	0.0001
FT x SR x IS	10	37.856	0.0001	25.825	0.0001
Med x IS	2	43.880	0.0016	27.127	0.0054
FT x Med x IS	10	14.661	0.0181	7.231	0.1726
SR x Med x IS	2	33.637	0.0071	21.137	0.0169
FT x SR x Med x IS	10	16.814	0.0064	7.804	0.1286
Position (Pos)	1	0.694	0.7478	5.252	0.3118
FT x Pos	5	3.715	0.7353	3.064	0.7010
SR x Pos	1	8.507	0.2608	12.543	0.1184
FT x SR x Pos	5	16.111	0.0367	4.106	0.5487
Med x Pos	1	4.340	0.4217	2.127	0.5196
FT x Med x Pos	5	0.903	0.9843	4.523	0.4921
SR x Med x Pos	1	6.250	0.3350	3.516	0.4078
FT x SR x Med x Pos	5	0.729	0.9904	1.745	0.8880
IS x Pos	2	2.127	0.7285	8.637	0.1865
FT x IS x Pos	10	10.929	0.0965	3.012	0.8235
SR x IS x Pos	2	4.731	0.4946	13.064	0.0793
FT x SR x IS x Pos	10	16.554	0.0073	4.627	0.5296
Med x IS x Pos	2	5.773	0.4238	4.470	0.4184
FT x Med x IS x Pos	10	2.179	0.9743	7.491	0.1512
SR x Med x IS x Pos	2	2.474	0.6918	3.516	0.5038
FT x SR x Med x IS x Pos	10	2.109	0.9772	5.182	0.4322
Error(FT x SR x Med x IS x Pos)	360	6.707		5.119	
Corrected Total	575	15.862		9.614	

Table 14. Analysis of variance of data for the effect of fungicide treatment, sterility regime, medium, infection site and position on the percentage berry parts isolated from Merlot grape bunches that yielded *Botrytis cinerea* at different phenological stages in the 2000/2001 season

		Véraison		Harvest	
Source of Variation	**DF**	**MS**	**SL**	**MS**	**SL**
Replicate	3	10.576	0.1814	6.525	0.5975
Fungicide Treatment (FT)	5	80.182	0.0001	206.814	0.0001
Error(FT)	15	5.715		10.101	
Sterility Regime (SR)	1	206.641	0.0001	814.627	0.0001
FT x SR	5	13.307	0.0802	58.898	0.0028
Error(FT)	18	5.599		10.547	
Medium (Med)	1	2.127	0.4561	104.210	0.0051
FT x Med	5	4.002	0.3942	14.939	0.2954
SR x Med	1	0.043	0.9149	47.266	0.0520
FT x SR x Med	5	1.502	0.8450	3.411	0.9146
Error(FT x SR x Med)	36	3.747		11.704	
Infection Site (IS)	2	298.047	0.0001	554.731	0.0001
FT x IS	10	31.120	0.0001	88.116	0.0001
SR x IS	2	94.661	0.0001	322.179	0.0001
FT x SR x IS	10	8.359	0.1365	23.168	0.0025
Med x IS	2	7.075	0.2817	24.262	0.0556
FT x Med x IS	10	2.231	0.9459	2.648	0.9763
SR x Med x IS	2	1.606	0.7495	27.474	0.0381
FT x SR x Med x IS	10	2.595	0.9111	2.214	0.9880
Position (Pos)	1	12.543	0.1341	47.266	0.0177
FT x Pos	5	8.168	0.1995	15.078	0.1101
SR x Pos	1	3.516	0.4272	19.141	0.1304
FT x SR x Pos	5	2.891	0.7615	4.036	0.7878
Med x Pos	1	2.127	0.5368	12.543	0.2206
FT x Med x Pos	5	0.460	0.9949	6.398	0.5733
SR x Med x Pos	1	0.391	0.7912	1.085	0.7184
FT x SR x Med x Pos	5	1.641	0.9157	5.356	0.6671
IS x Pos	2	3.950	0.4924	3.516	0.6560
FT x IS x Pos	10	2.543	0.9167	8.984	0.3778
SR x IS x Pos	2	8.203	0.2303	0.130	0.9845
FT x SR x IS x Pos	10	4.297	0.6557	2.995	0.9630
Med x IS x Pos	2	6.033	0.3393	0.304	0.9642
FT x Med x IS x Pos	10	2.335	0.9370	4.939	0.8198
SR x Med x IS x Pos	2	4.036	0.4848	0.043	0.9948
FT x SR x Med x IS x Pos	10	2.630	0.9074	4.158	0.8904
Error(FT x SR x Med x IS x Pos)	360	5.564		8.330	
Corrected Total	575	7.918		16.764	

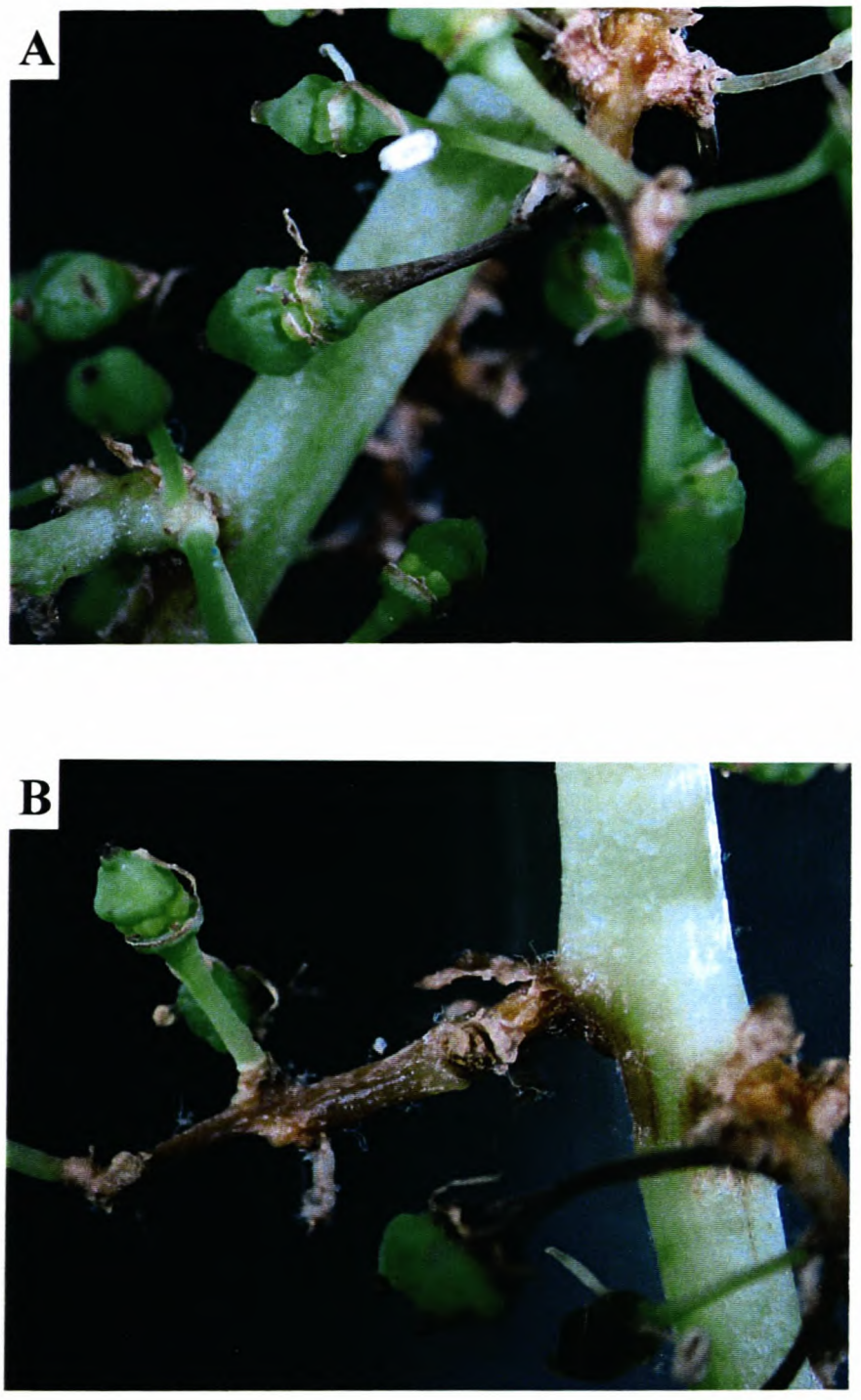

Fig. 1. *Botrytis cinerea* symptoms on unsprayed grape clusters (cultivar Merlot) inoculated with dry, airborne conidia of *Botrytis cinerea* at full bloom stage. **A.** Cluster showing withered filaments and decayed pedicels. Ovaries remained green. **B.** Clusters with withered filaments, decayed pedicels and laterals. Some pedicels, and all the ovaries remained green.

Fig. 2. Grape clusters (cultivar Merlot) sprayed with fungicides and inoculated with dry, airborne conidia of *Botrytis cinerea* at full bloom stage. **A.** Cluster sprayed with cyprodinil/fludioxonil showing asymptomatic calyptra, filaments, ovaries, and pedicels. **B.** Cluster sprayed with pyrimethanil showing asymptomatic filaments, ovaries, pedicels and lateral.